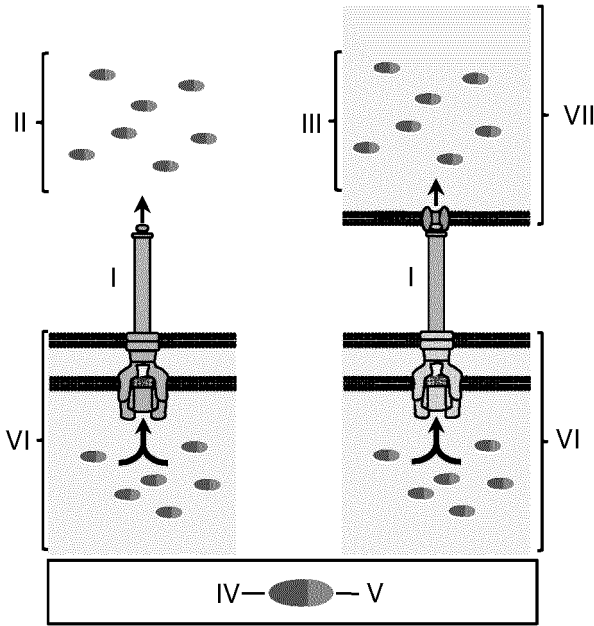


Figure 1

A



B

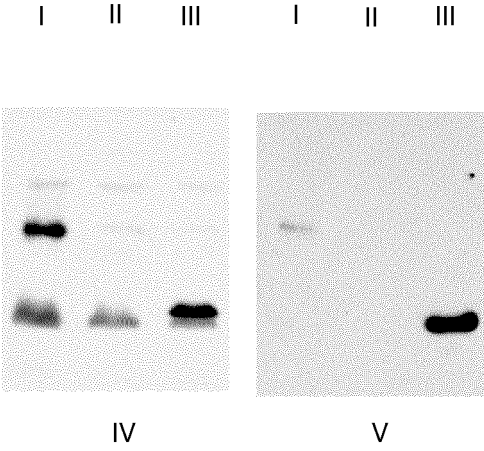


Figure 2

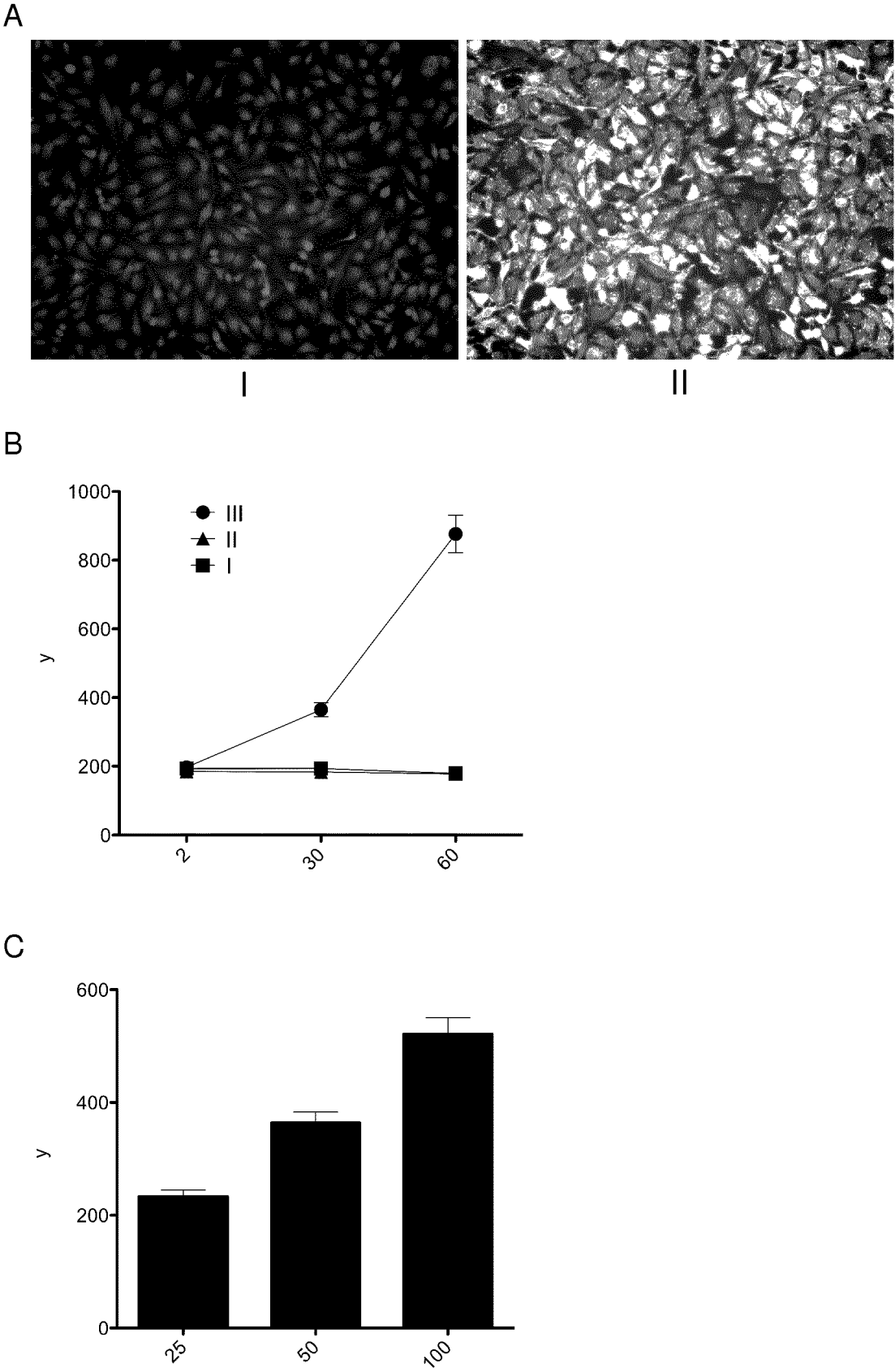


Figure 3

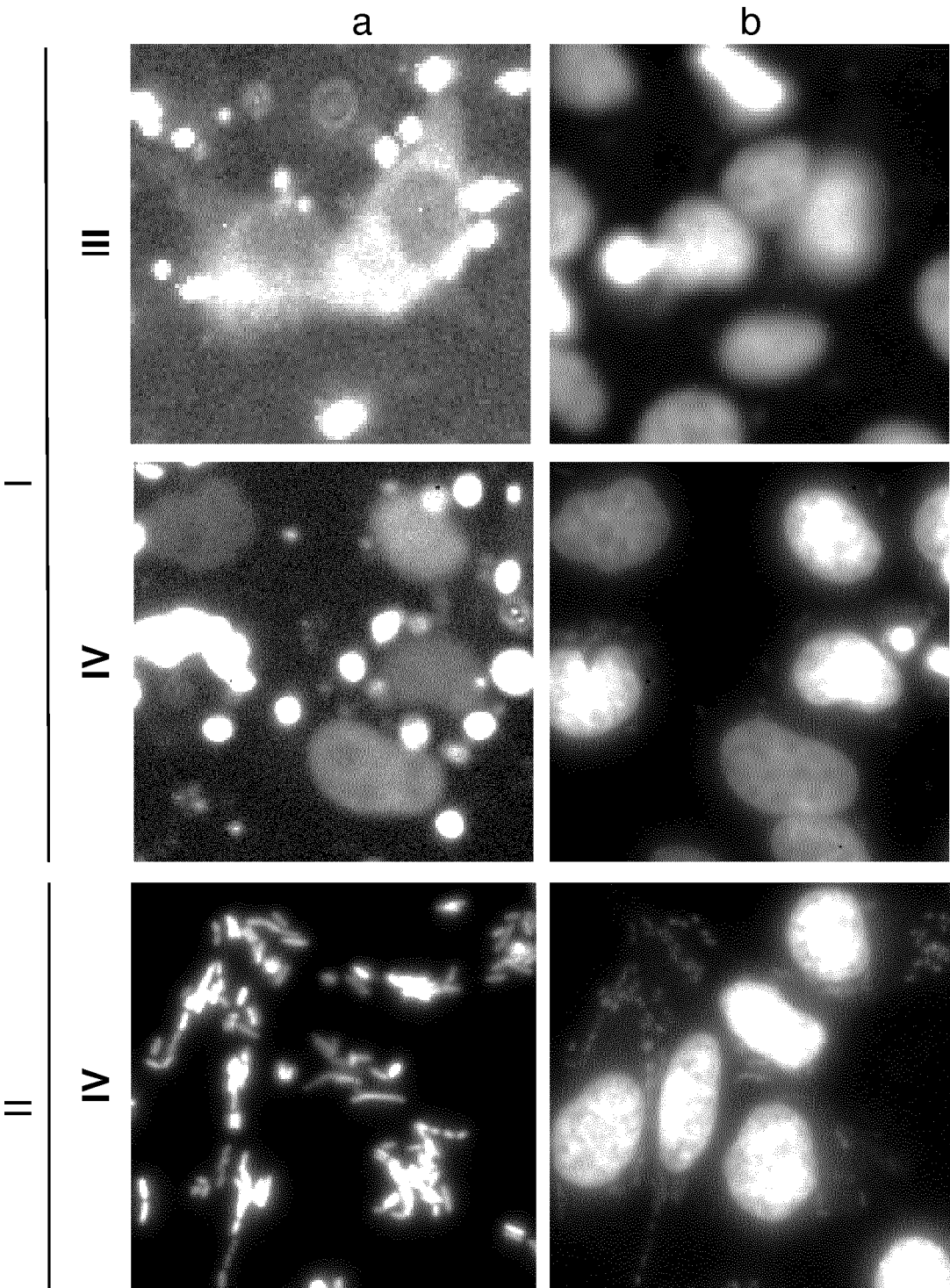


Figure 4

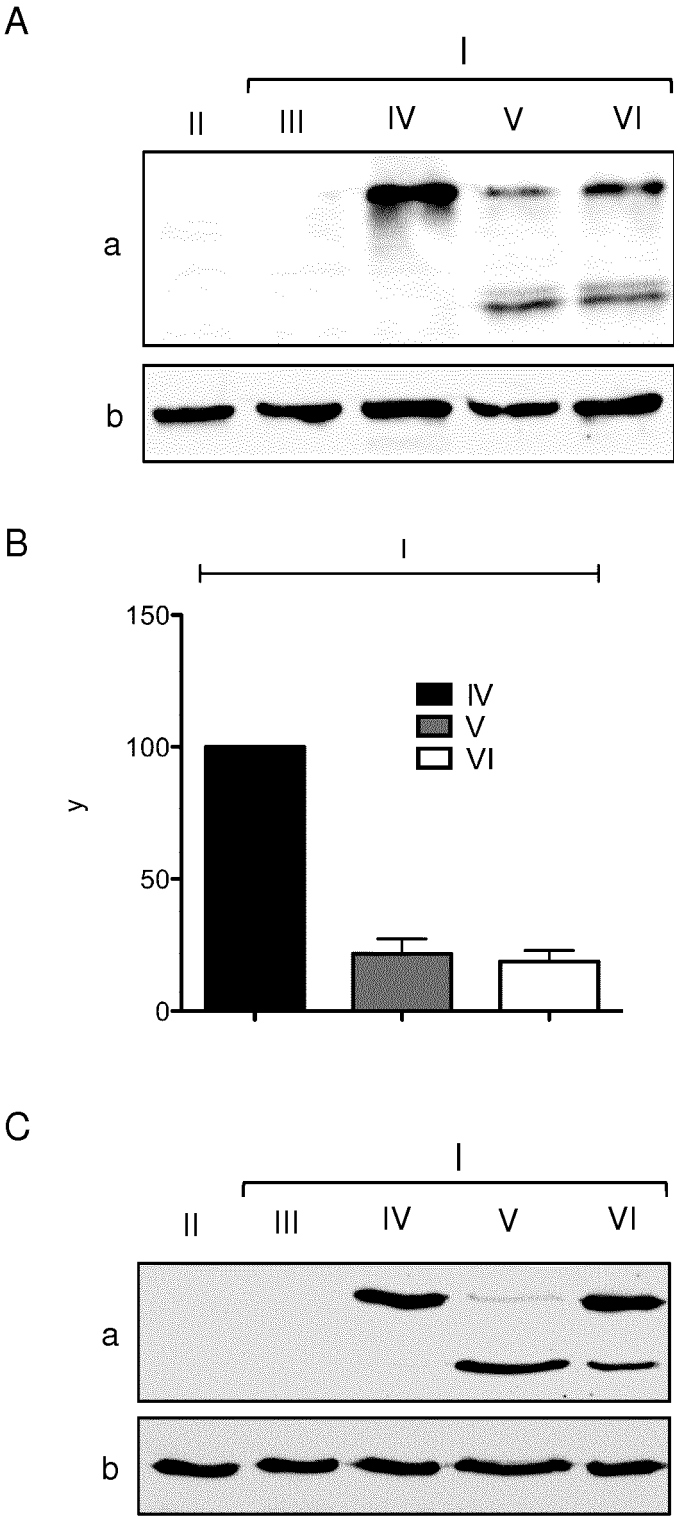


Figure 5

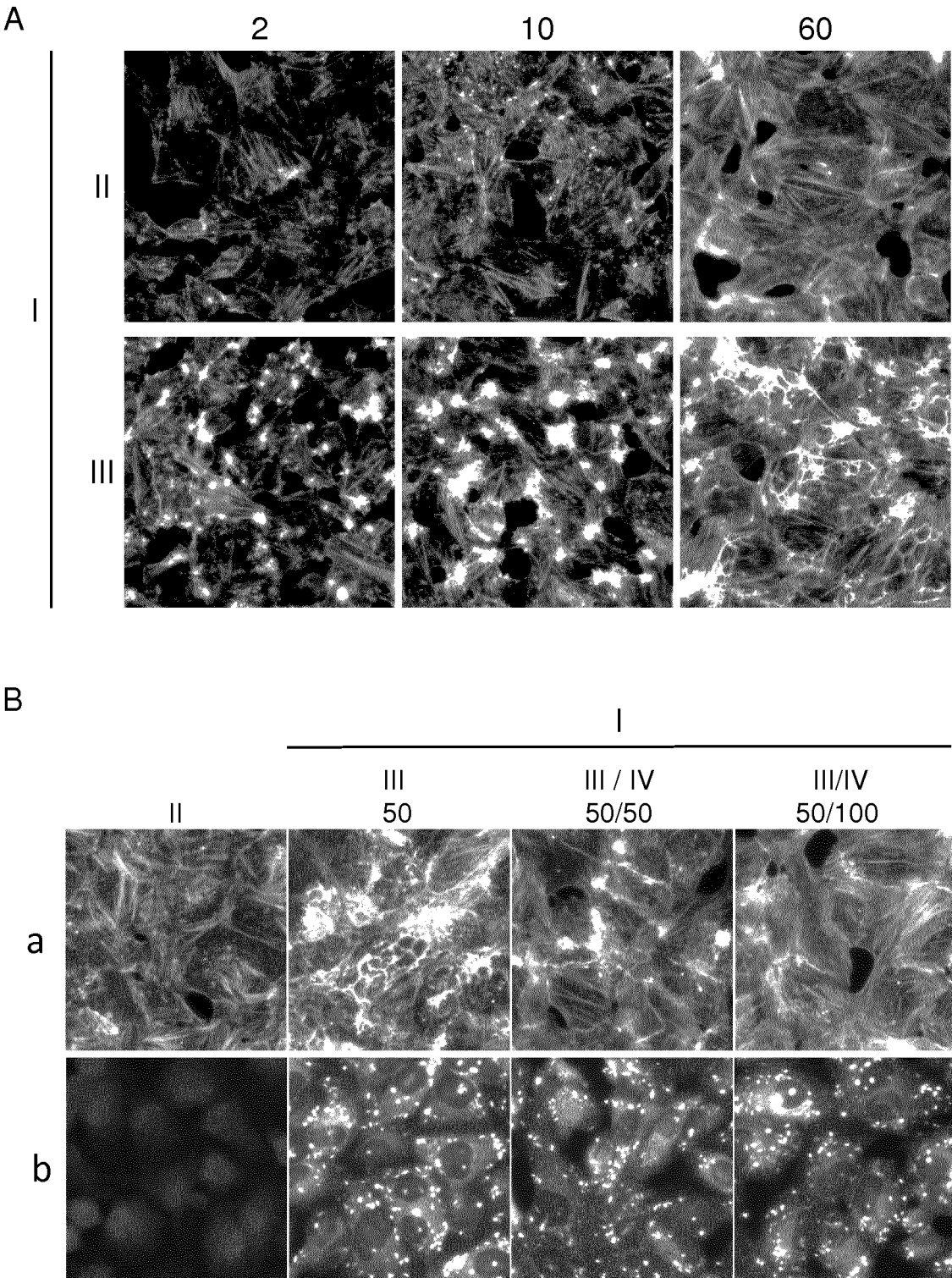


Figure 6

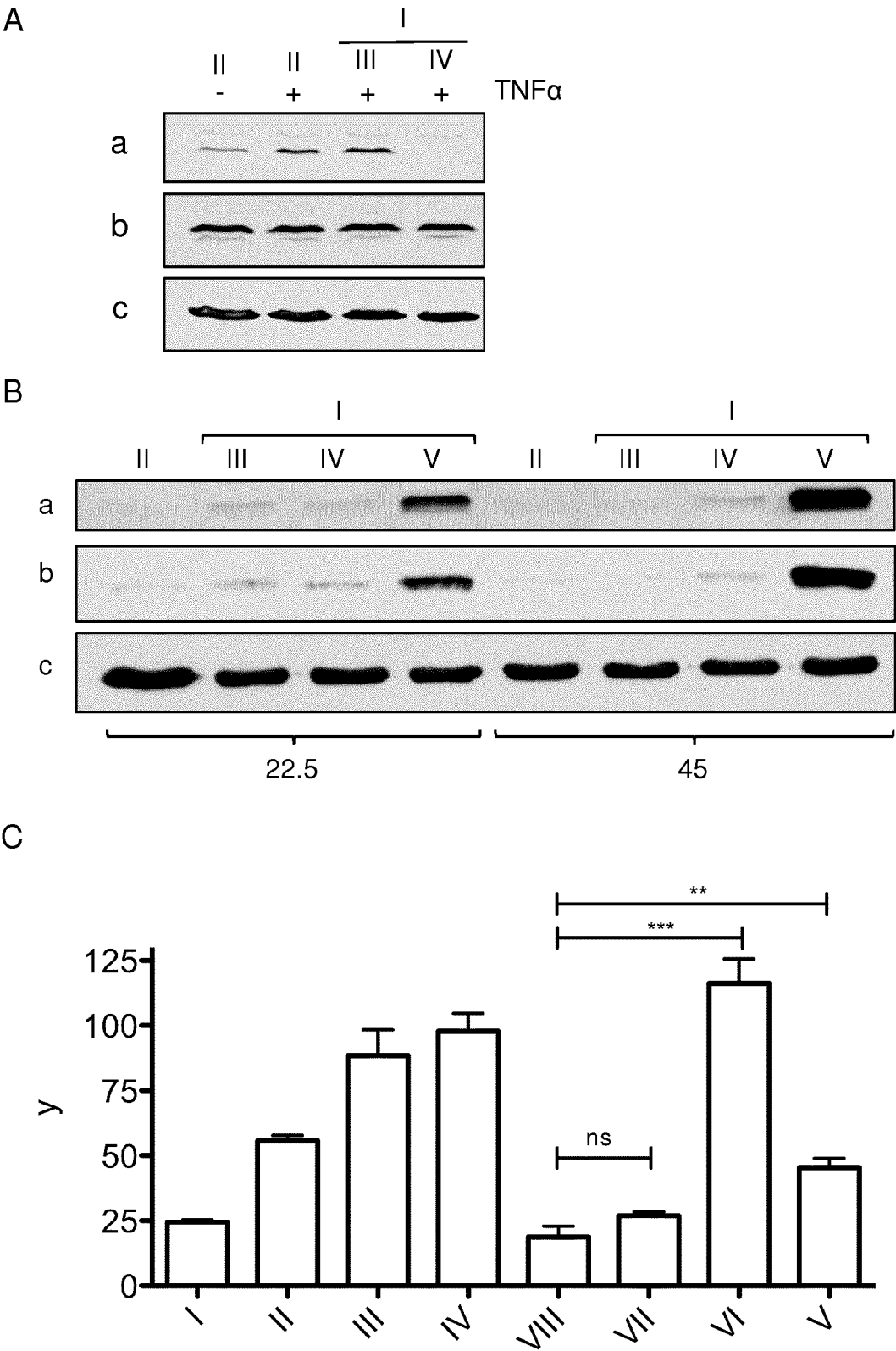


Figure 7

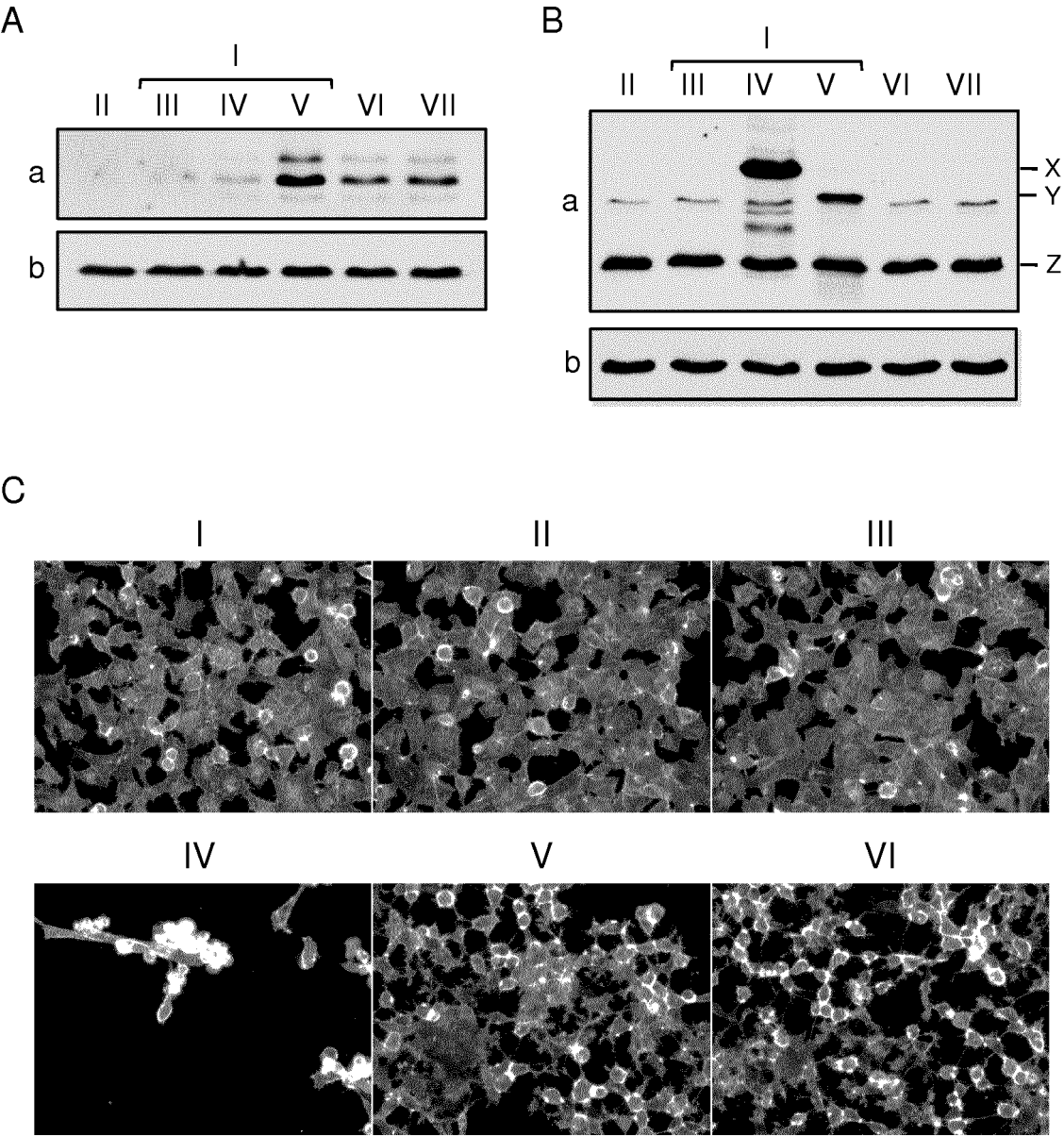


Figure 8

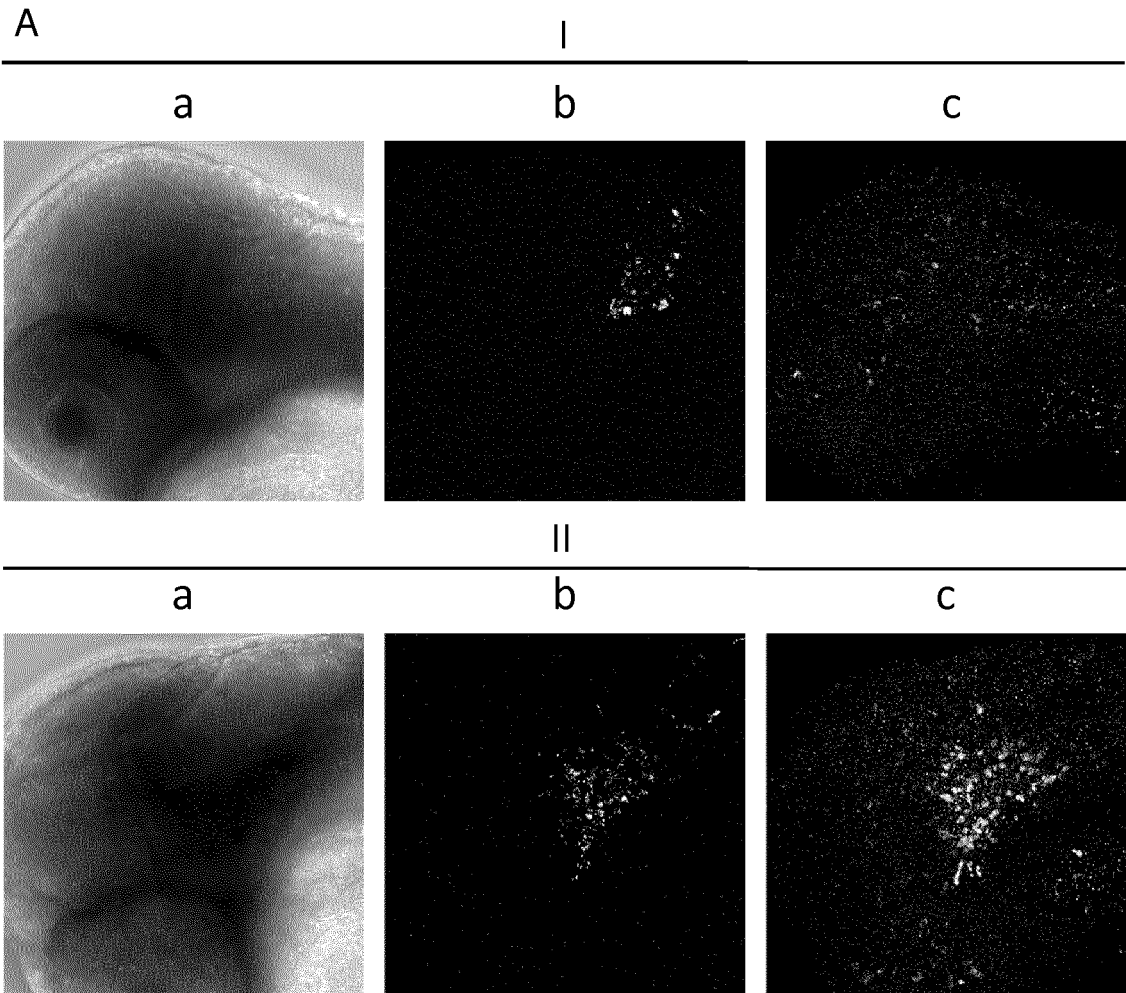
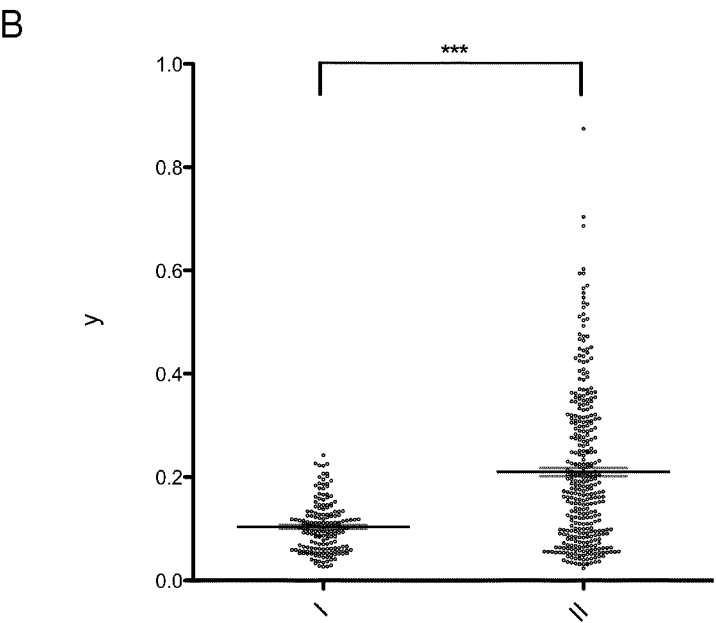
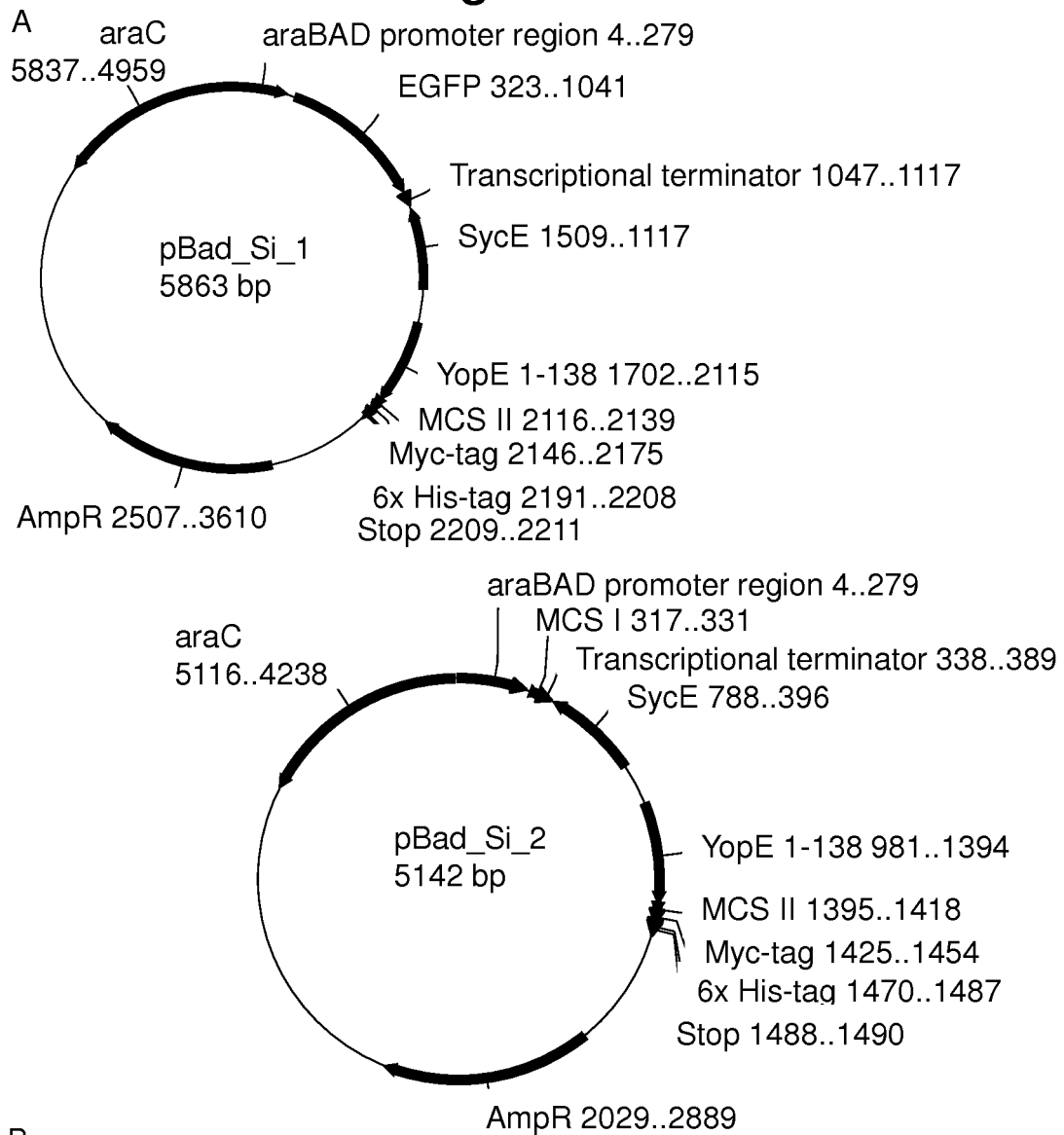


Figure 10

YopE1-138 XhoI XbaI BstBI
 GTTCGCCACG CTCGAGTCTA GATTGAA
 HindIII myc epitope
 AAGCTTGGGC CCGAACAAAACTCATCTCA

GAAGAGGATCTGAATAGCGC CGTCGACCAT
 6x His - tag ★★★
 CATCATCATC ATCATTGAGT TTAAACGGTC

TCCAGCTTGG CTGTTTTGG C

Figure 11

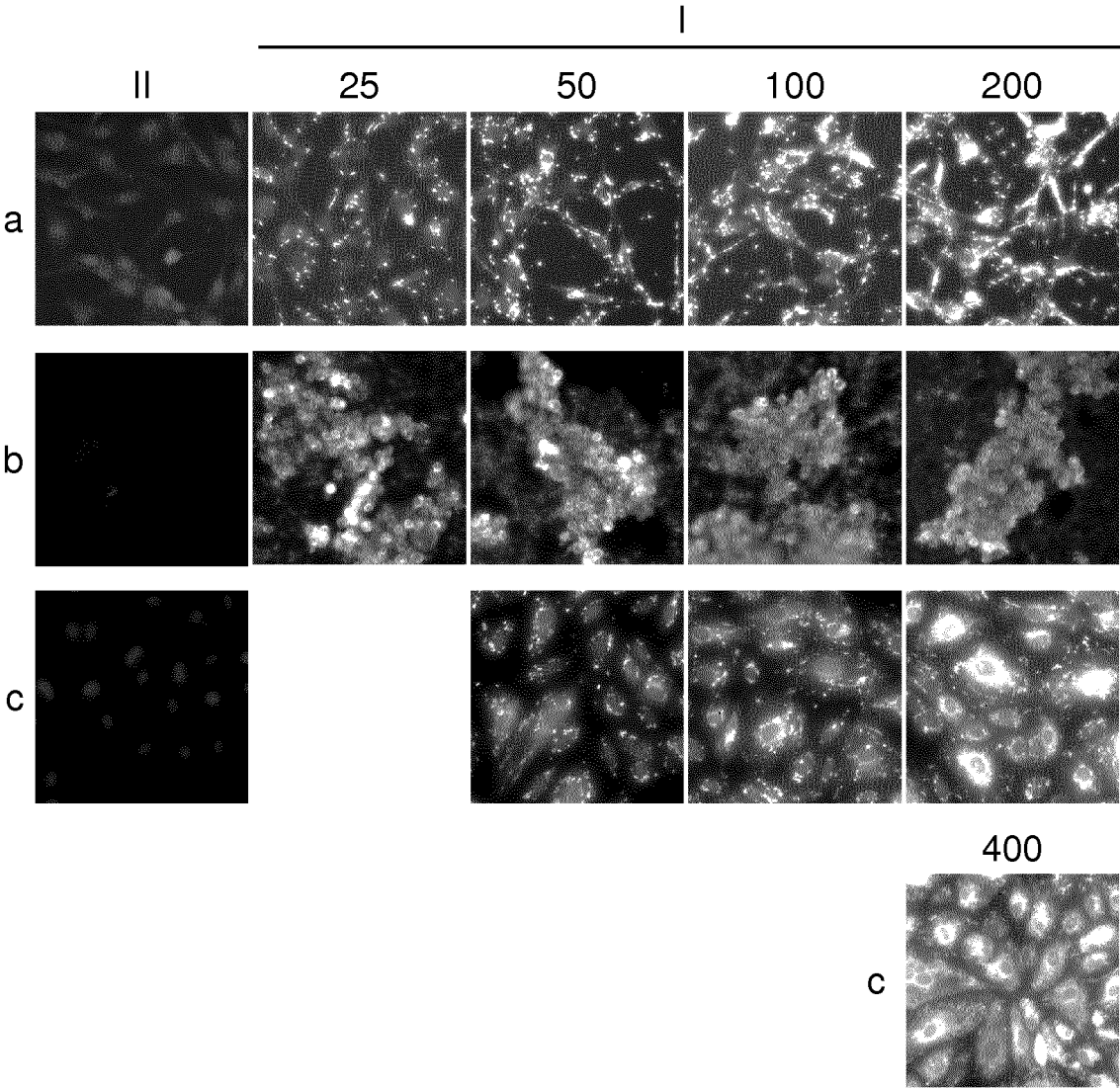


Figure 12

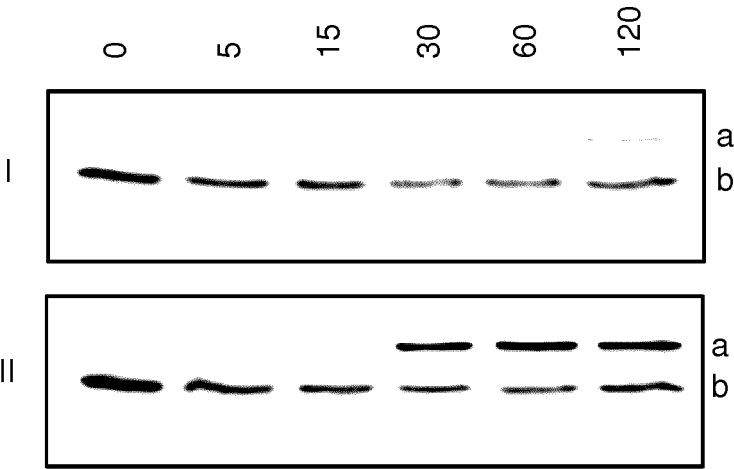
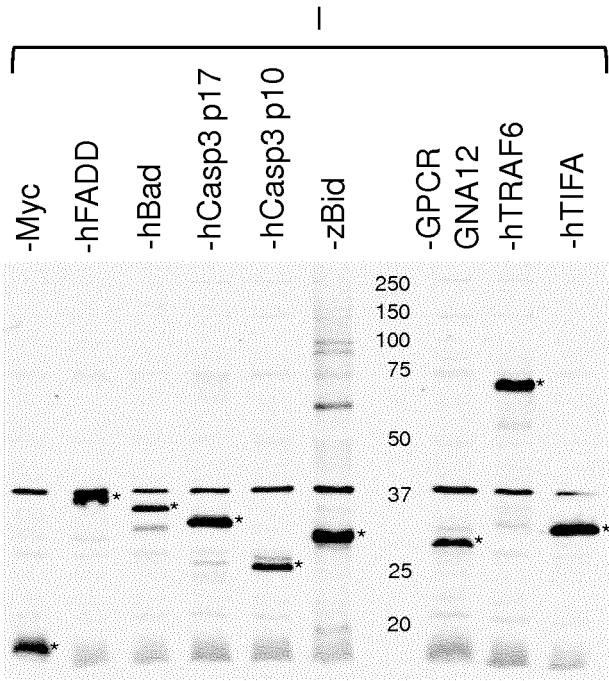


Figure 13

A



B

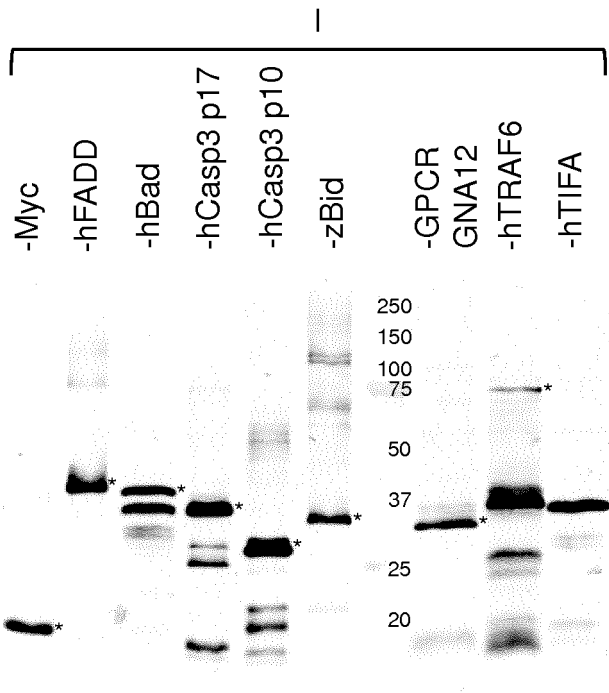
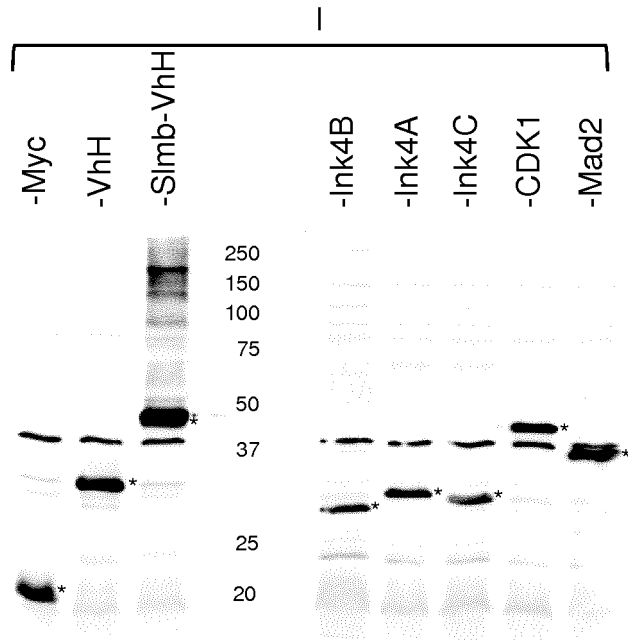


Figure 14

A



B

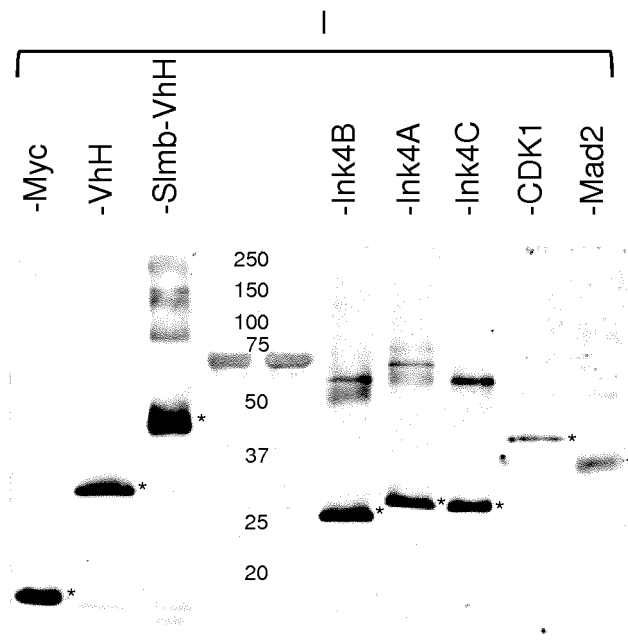


Figure 15A

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T	MRS40 pIML421 [yopH Δ 1-352, yopO Δ 65-558, yopP23, yopE21, yopM23, yopT135]					Nal	Iriarte and Cornelis, 1998
Δ HOPEMT asd yopB	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ yopB	MRS40 pIML421 [yopB Δ 89-217, yopH Δ 1-352, yopO Δ 65-558, yopP23, yopE21, yopM23, yopT135]					Nal Kan	
Δ HOPEMT asd	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd	MRS40 asd Δ 292-610 pIML421 [yopH Δ 1-352, yopO Δ 65-558, yopP23, yopE21, yopM23, yopT135]					Nal	Kudryashev et al., 2013
Δ HOPEMT asd inv	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd Δ invA	MRS40 asd Δ 292-610 invA Δ 352-2225::aphA-3 pIML421 [yopH Δ 1-352, yopO Δ 65-558, yopP23, yopE21, yopM23, yopT135]				445/446, 447/448, 449/450	Nal Kan	
Δ HOPEMT asd inv yadA	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd Δ invA Δ yadA	MRS40 asd Δ 292-610 invA Δ 587-836 (vector cointegration) yadA Δ 89-354::aphA3 pIML421 [yopH Δ 1-352, yopO Δ 65-558, yopP23, yopE21, yopM23, yopT135]				352/353, 354/355, 356/357	Nal Kan Tet	

Figure 15B

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pBad_Si1	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd	EGFP (Arabinose inducible), SycE-YopE1-138-MychHis fragment		pBad- MychHisA (Invitrogen)	pBad_Si_1	285/286 (EGFP), 287/288 (sycE- YopE1- 138)	Nal Amp	
Δ HOPEMT asd pBad_Si2	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd	SycE-YopE1-138-MychHis fragment	YopE1- 138- MychHis	pBad- MychHisA (Invitrogen)	pBad_Si_2	287/288 (sycE- YopE1- 138)	Nal Amp	
Δ HOPEMT asd pSi_16	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd		YopE1- 138- lpgB1	pBad_Si_2	pSi_16	292/293	Nal Amp	
Δ HOPEMT asd pSi_20	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd		YopE1- 138- SopE	pBad_Si_2	pSi_20	296/297	Nal Amp	
Δ HOPEMT asd pSi_22	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd		YopE1- 138- Rac1 Q61L	pBad_Si_2	pSi_22	299/300	Nal Amp	
Δ HOPEMT asd pSi_24	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd		YopE1- 138- RhoA Q61E	pBad_Si_2	pSi_24	301/302	Nal Amp	

Figure 15C

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEM T asd pSi_28	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-SopE-MycHis	pBad_Si_2	pSi_28	296/306	Nal Amp	
Δ HOPEM T yopB asd pSi_28	Y. enterocolitica Δ yopH,O,P,E,M,T Δ yopB Δ asd		YopE1-138-SopE-MycHis	pBad_Si_2	pSi_28	296/306	Nal Amp	
Δ HOPEM T asd pSi_30	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-SopB	pBad_Si_2	pSi_30	307/308	Nal Amp	
Δ HOPEM T asd pSi_37	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-FADD	pBad_Si_2	pSi_37	367/386	Nal Amp	
Δ HOPEM T asd pSi_38	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-OspF	pBad_Si_2	pSi_38	317/318	Nal Amp	
Δ HOPEM T asd pSi_43	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-BepG 715-end	pBad_Si_2	pSi_43	324/351	Nal Amp	

Figure 15D

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_51	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Rac1 Q61L- MycHis	pBad_Si_2	pSi_51	299/339	Nal Amp	
Δ HOPEMT yopB asd pSi_51	Y. enterocolitica Δ yopH,O,P,E,M,T Δ yopB Δ asd		YopE1-138-Rac1 Q61L- MycHis	pBad_Si_2	pSi_51	299/339	Nal Amp	
Δ HOPEMT asd pSi_53	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Slmb1- VhH4	pBad_Si_2	pSi_53	341/342	Nal Amp	
Δ HOPEMT asd pSi_57	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Bad	pBad_Si_2	pSi_57	346/347	Nal Amp	
Δ HOPEMT asd pSi_64	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-SptP	pBad_Si_2	pSi_64	364/365	Nal Amp	
Δ HOPEMT asd pSi_70	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-NLS-Slmb1- VhH4	pBad_Si_2	pSi_70	369/342	Nal Amp	

Figure 15E

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_85	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Bid	pBad_Si_2	pSi_85	387/391	Nal Amp	
Δ HOPEMT asd pSi_87	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-t-Bid	pBad_Si_2	pSi_87	389/391	Nal Amp	
Δ HOPEMT asd pSi_97	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Caspase3 p17	pBad_Si_2	pSi_97	403/406	Nal Amp	
Δ HOPEMT asd pSi_103	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-GPCR GNA12	pBad_Si_2	pSi_103	410/413	Nal Amp	
Δ HOPEMT asd pSi_106	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Caspase3 p10/12	pBad_Si_2	pSi_106	417/420	Nal Amp	
Δ HOPEMT asd pSi_111	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-lpgD	pBad_Si_2	pSi_111	423/424	Nal Amp	
Δ HOPEMT asd pSi_112	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Slmb1- VhH4-NLS	pBad_Si_2	pSi_112	341/425	Nal Amp	

Figure 15F

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_116	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-z-Bid	pBad_Si_2	pSi_116	428/430	Nal Amp	
Δ HOPEMT asd pSi_117	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-z-t-Bid	pBad_Si_2	pSi_117	429/430	Nal Amp	
Δ HOPEMT asd pSi_118	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-BepA E305- end	pBad_Si_2	pSi_118	433/435	Nal Amp	
Δ HOPEMT asd pSi_119	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-BepA	pBad_Si_2	pSi_119	434/435	Nal Amp	
Δ HOPEMT asd pSi_120	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-ET1	pBad_Si_2	pSi_120	436/437	Nal Amp	
Δ HOPEMT asd pSi_121	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-z-BIM	pbad_Si_1	pSi_121	438/439	Nal Amp	
Δ HOPEMT asd pSi_124	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M, T Δ asd		YopE1-138-VhH4 nanobody recognizing EGFP	pBad_Si_2	pSi_124	451/373	Nal Amp	

Figure 15G

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_132	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-TEV protease S219V	pBad_Si_2	pSi_132	463/464	Nal Amp	
Δ HOPEMT asd pSi_140	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-EGFP	pBad_Si_2	pSi_140	477/476	Nal Amp	
Δ HOPEMT asd pSi_143	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-Cdk1	pBad_Si_2	pSi_143	478/479	Nal Amp	
Δ HOPEMT asd pSi_145	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-Mad2	pBad_Si_2	pSi_145	482/483	Nal Amp	
Δ HOPEMT asd pSi_147	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-Ink4A	pBad_Si_2	pSi_147	486/487	Nal Amp	
Δ HOPEMT asd pSi_150	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-Ink4B	pBad_Si_2	pSi_150	492/493	Nal Amp	
Δ HOPEMT asd pSi_151	Y. enterocolitica Δ yopH,O,P,E,M, T Δ asd		YopE1-138-Ink4C	pBad_Si_2	pSi_151	494/495	Nal Amp	

Figure 15H

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_153	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-TIFA	pBad_Si_2	pSi_153	558/559	Nal Amp	
Δ HOPEMT asd pSi_156	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-2x TEVsite - ET1	pBad_Si_2	pSi_156	504/505	Nal Amp	
Δ HOPEMT asd pSi_159	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138- 2xTEVsite - EGFP - NLS	pBad_Si_2	pSi_159	511/513	Nal Amp	
Δ HOPEMT asd pSi_160	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138- 2xTEVsite - NLS - EGFP	pBad_Si_2	pSi_160	512/476	Nal Amp	
Δ HOPEMT asd pSi_161	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-2x TEVsite - INK4C	pBad_Si_2	pSi_161	508/509	Nal Amp	
Δ HOPEMT asd pSi_164	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-2x TEVsite - Flag - INK4C	pBad_Si_2	pSi_164	515/509	Nal Amp	
Δ HOPEMT asd pSi_166	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-murine Traf6	pBad_Si_2	pSi_166	561/562	Nal Amp	

Figure 15I

Strain Name	Background strain	Insert/rel evant character istics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resist ances	Referenc e
Δ HOPEMT asd pSi_318	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized murine tBid BH3 part	pBad_Si_2	pSi_318	677/678	Nal Amp	
Δ HOPEMT asd pSi_322	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized murine Bax BH3 part	pBad_Si_2	pSi_322	682/683	Nal Amp	
S. enterica SL1344 Δ aroA	S. enterica subsp. enterica serovar Typhimurium SL1344 Δ aroA							Hoiseth and Stocker, 1981
S. enterica Δ aroA pSi_266	S. enterica SL1344 Δ aroA		SteA1-20	pBad- MycHisA (Invitrogen)	pSi_266	580/612	Amp	
S. enterica Δ aroA pSi_267	S. enterica SL1344 Δ aroA		SteA	pBad- MycHisA (Invitrogen)	pSi_267	580/613	Amp	

Figure 15J

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
S. enterica Δ aroA pSi_268	S. enterica SL1344 Δ aroA		SopE1-80	pBad-MycHisA (Invitrogen)	pSi_268	614/615	Amp	
S. enterica Δ aroA pSi_269	S. enterica SL1344 Δ aroA		SopE1-104	pBad-MycHisA (Invitrogen)	pSi_269	614/616	Amp	
S. enterica Δ aroA pSi_270	S. enterica SL1344 Δ aroA		SteA1-20-S. enterica codon optimized murine tBid	pSi_266	pSi_270	synthetic construct	Amp	
S. enterica Δ aroA pSi_271	S. enterica SL1344 Δ aroA		SteA-S. enterica codon optimized murine tBid	pSi_267	pSi_271	synthetic construct	Amp	
S. enterica Δ aroA pSi_272	S. enterica SL1344 Δ aroA		SopE1-80-S. enterica codon optimized murine tBid	pSi_268	pSi_272	synthetic construct	Amp	
S. enterica Δ aroA pSi_273	S. enterica SL1344 Δ aroA		SopE1-104-S. enterica codon optimized murine tBid	pSi_269	pSi_273	synthetic construct	Amp	

Figure 15K

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistance	Reference
Δ HOPEMT asd pSi_362	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized Ink4A 84-103	pBad_Si_2	pSi_362	745/746	Nal Amp	
Δ HOPEMT asd pSi_363	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized Cdk2/cyclin inhibitory peptide I	pBad_Si_2	pSi_363	747/748	Nal Amp	
Δ HOPEMT asd pSi_364	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized p21 peptide 10 (DtoA)	pBad_Si_2	pSi_364	749/750	Nal Amp	
Δ HOPEMT asd pSi_366	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized p21 core of peptide 10 and 11	pBad_Si_2	pSi_366	753/754	Nal Amp	
Δ HOPEMT asd pSi_367	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized p21 peptide 17-33	pBad_Si_2	pSi_367	755/756	Nal Amp	

Figure 15L

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_368	<i>Y. enterocolitica</i> Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. <i>enterocolitica</i> codon optimized pep5 of G1/S cyclin D2	pBad_Si_2	pSi_368	757/758	Nal Amp	
<i>S. enterica</i> Δ aroA pSi_333	<i>S. enterica</i> SL1344 Δ aroA		SteA-Ink4a-MycHis	pSi_267	pSi_333	703/704	Amp	
<i>S. enterica</i> Δ aroA pSi_334	<i>S. enterica</i> SL1344 Δ aroA		SopE1-104-Ink4a-MycHis	pSi_269	pSi_334	703/704	Amp	
<i>S. enterica</i> Δ aroA pSi_335	<i>S. enterica</i> SL1344 Δ aroA		SteA-Ink4c-MycHis	pSi_267	pSi_335	705/706; 707/708; 705/708	Amp	
<i>S. enterica</i> Δ aroA pSi_336	<i>S. enterica</i> SL1344 Δ aroA		SopE1-104-Ink4c-MycHis	pSi_269	pSi_336	705/706; 707/708; 705/708	Amp	
<i>S. enterica</i> Δ aroA pSi_337	<i>S. enterica</i> SL1344 Δ aroA		SteA-Mad2-MycHis	pSi_267	pSi_337	709/710	Amp	

Figure 15M

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
S. enterica Δ aroA pSi_338	S. enterica SL1344 Δ aroA		SopE1-104-Mad2-MycHis	pSi_269	pSi_338	709/710	Amp	
S. enterica Δ aroA pSi_339	S. enterica SL1344 Δ aroA		SteA-Cdk1-MycHis	pSi_267	pSi_339	711/712	Amp	
S. enterica Δ aroA pSi_340	S. enterica SL1344 Δ aroA		SopE1-104-Cdk1-MycHis	pSi_269	pSi_340	711/712	Amp	
Δ HOPEMT asd pSi_315	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized murine tBid	pBad_Si_2	pSi_315	synthetic construct	Nal Amp	
Δ HOPEMT asd pSi_236	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Ubiquitin	pBad_Si_2	pSi_236	585/586	Nal Amp	
Δ HOPEMT asd pSi_237_II	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Ubiquitin-Flag-INK4C-MycHis	pSi_236	pSi_237_II	588/509	Nal Amp	

Figure 15N

Strain Name	Background strain	Insert/relevant characteristics	Protein to be delivered by T3SS	Backbone plasmid	Resulting plasmid name	Primers. Si_Nr.:	resistances	Reference
Δ HOPEMT asd pSi_357	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized murine tBid BH3	pBadSi_2	pSi_357	733/735	Nal Amp	
Δ HOPEMT asd pSi_358	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-Y. enterocolitica codon optimized murine Bax BH3	pBad_Si_2	pSi_358	736/738	Nal Amp	
Δ HOPEMT asd pSi_371	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-(Y. enterocolitica codon optimized murine tBid BH3)2	pSi_357	pSi_371	733/734	Nal Amp	
Δ HOPEMT asd pSi_373	Y. enterocolitica Δ yopH,O,P,E,M,T Δ asd		YopE1-138-(Y. enterocolitica codon optimized murine tBid BH3)- (Y. enterocolitica codon optimized murine Bax BH3)	pSi_358	pSi_373	733/734	Nal Amp	

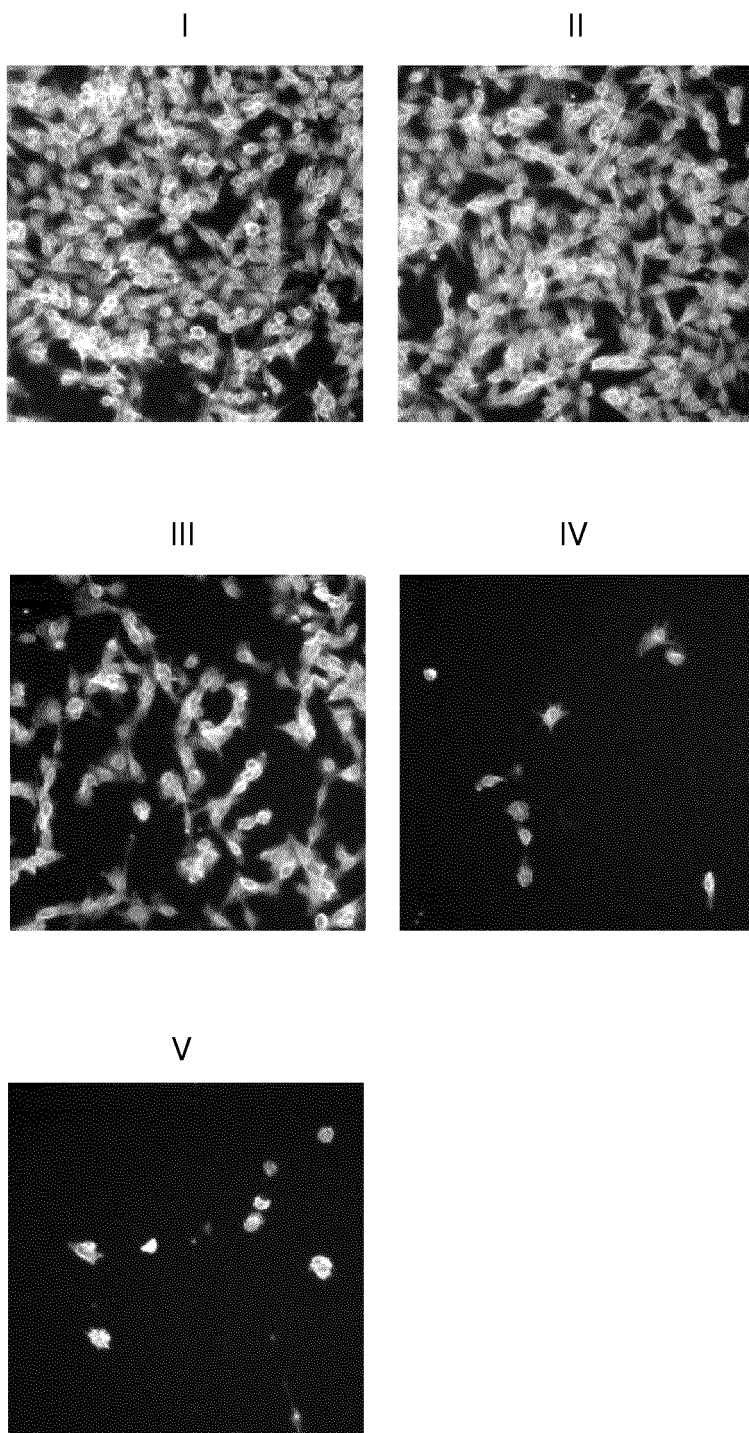
Figure 16

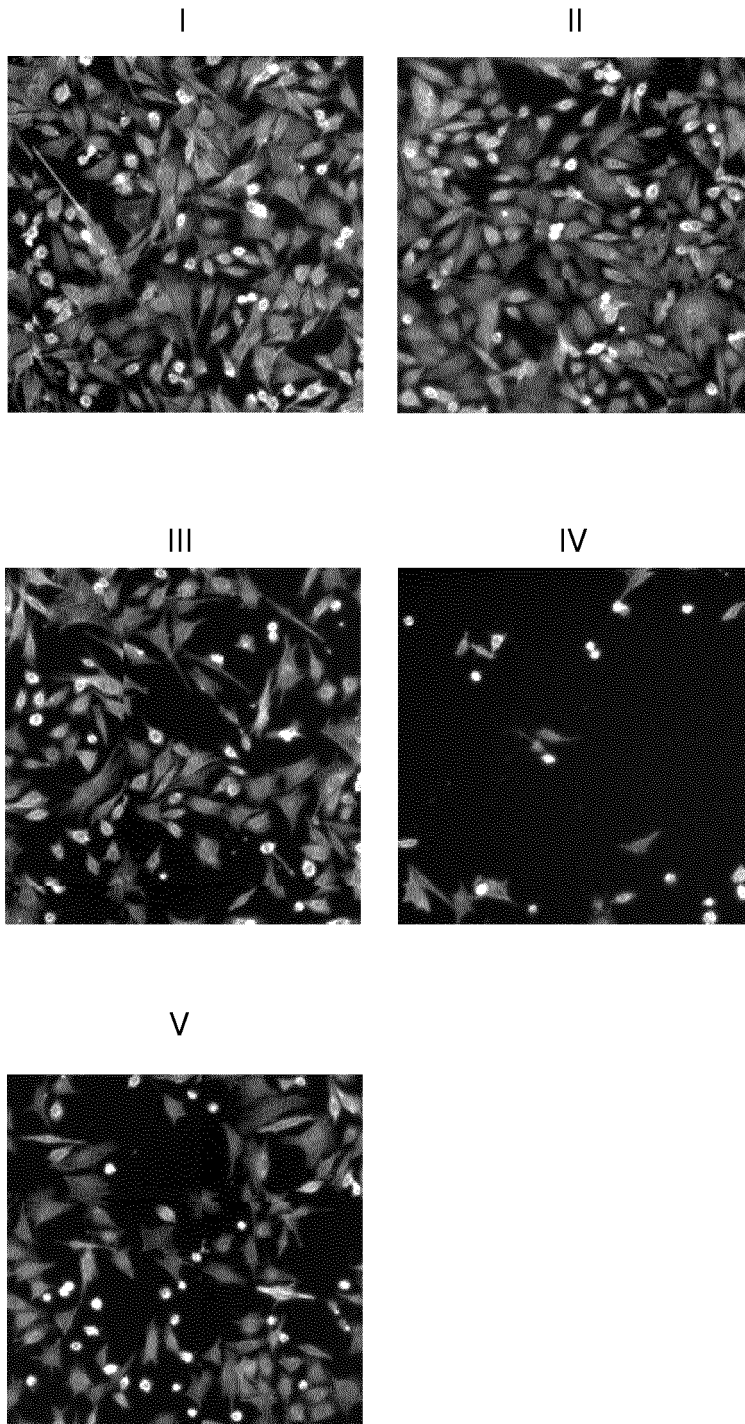
Figure 17

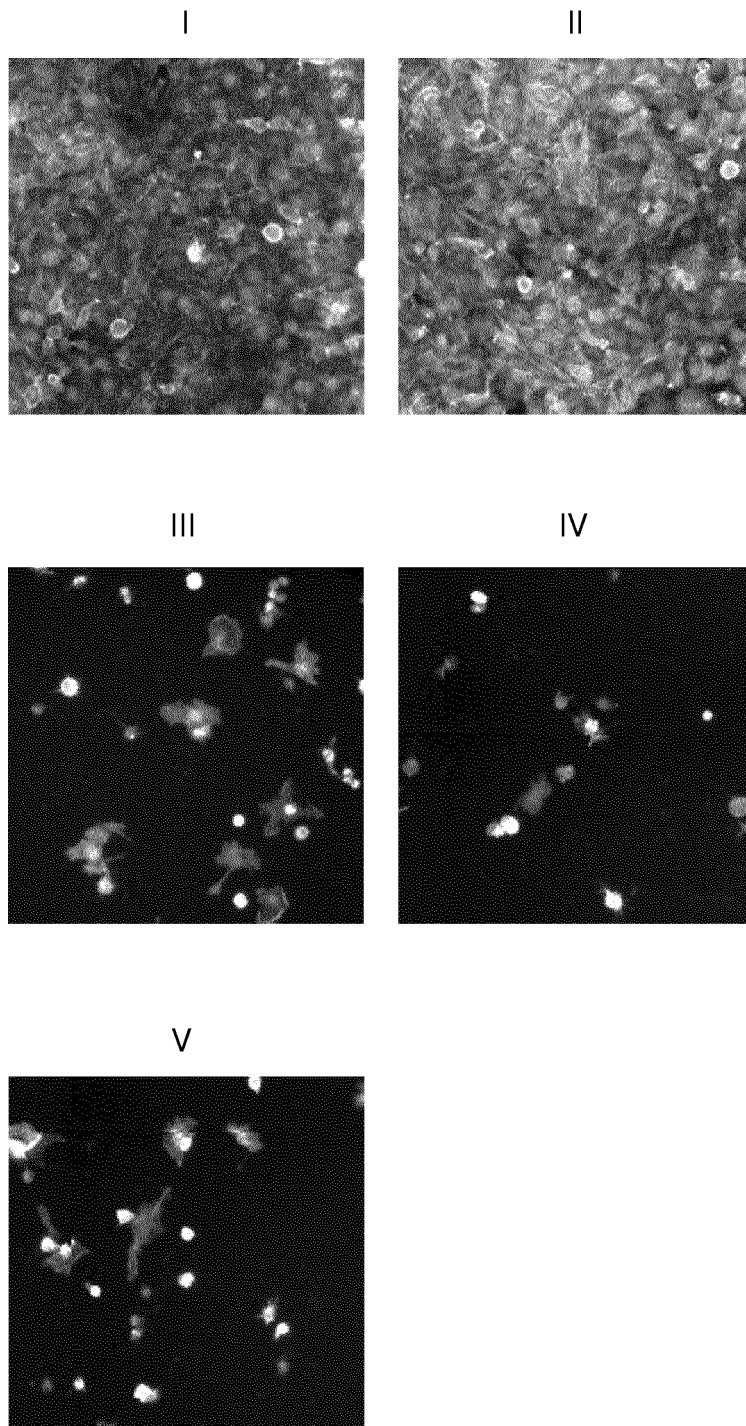
Figure 18

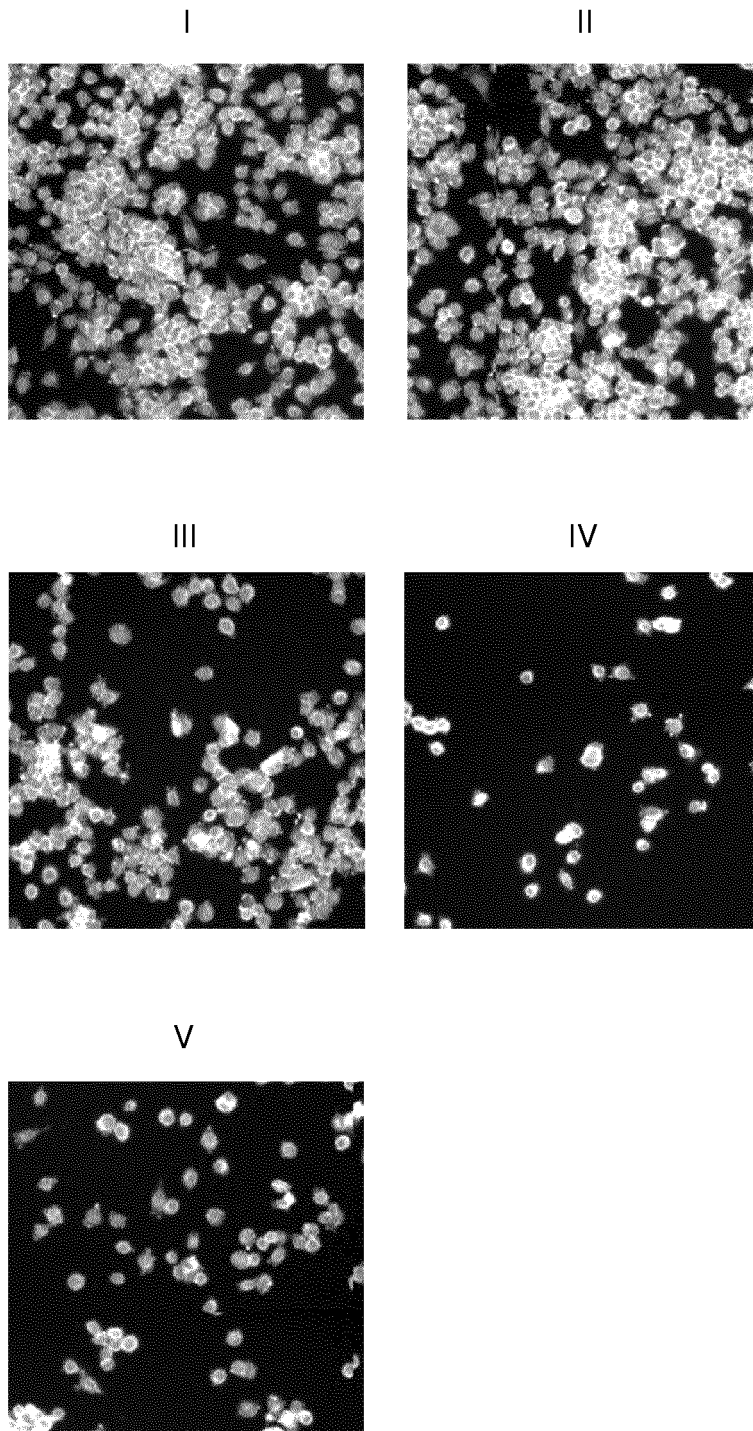
Figure 19

Figure 20

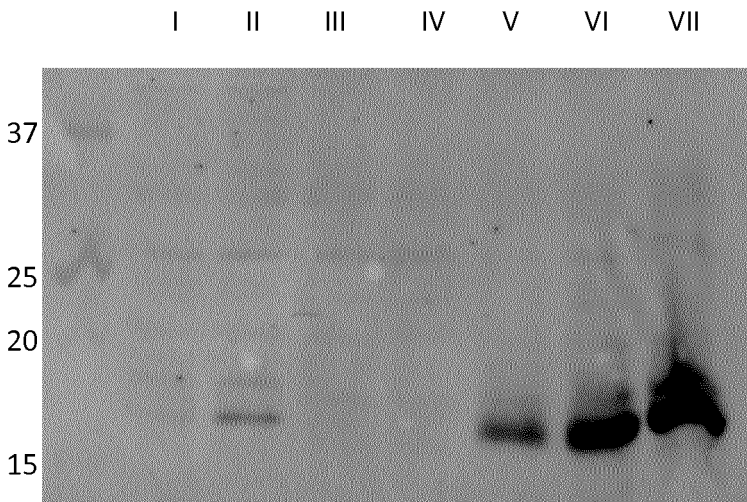


Figure 21

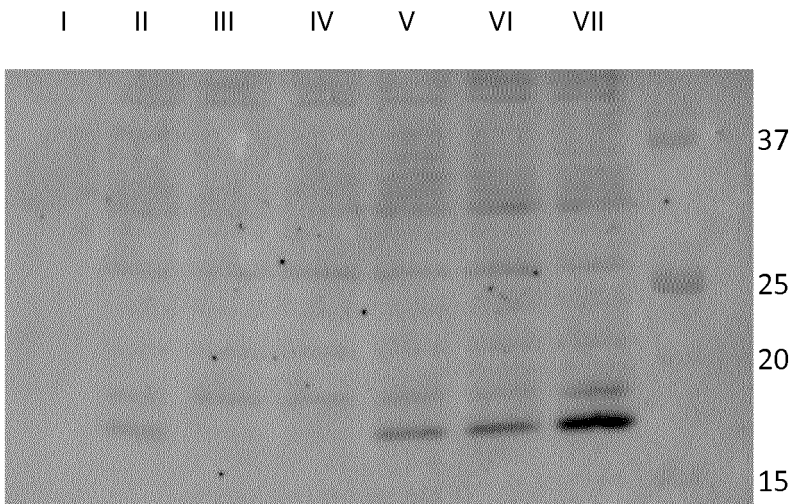


Figure 22

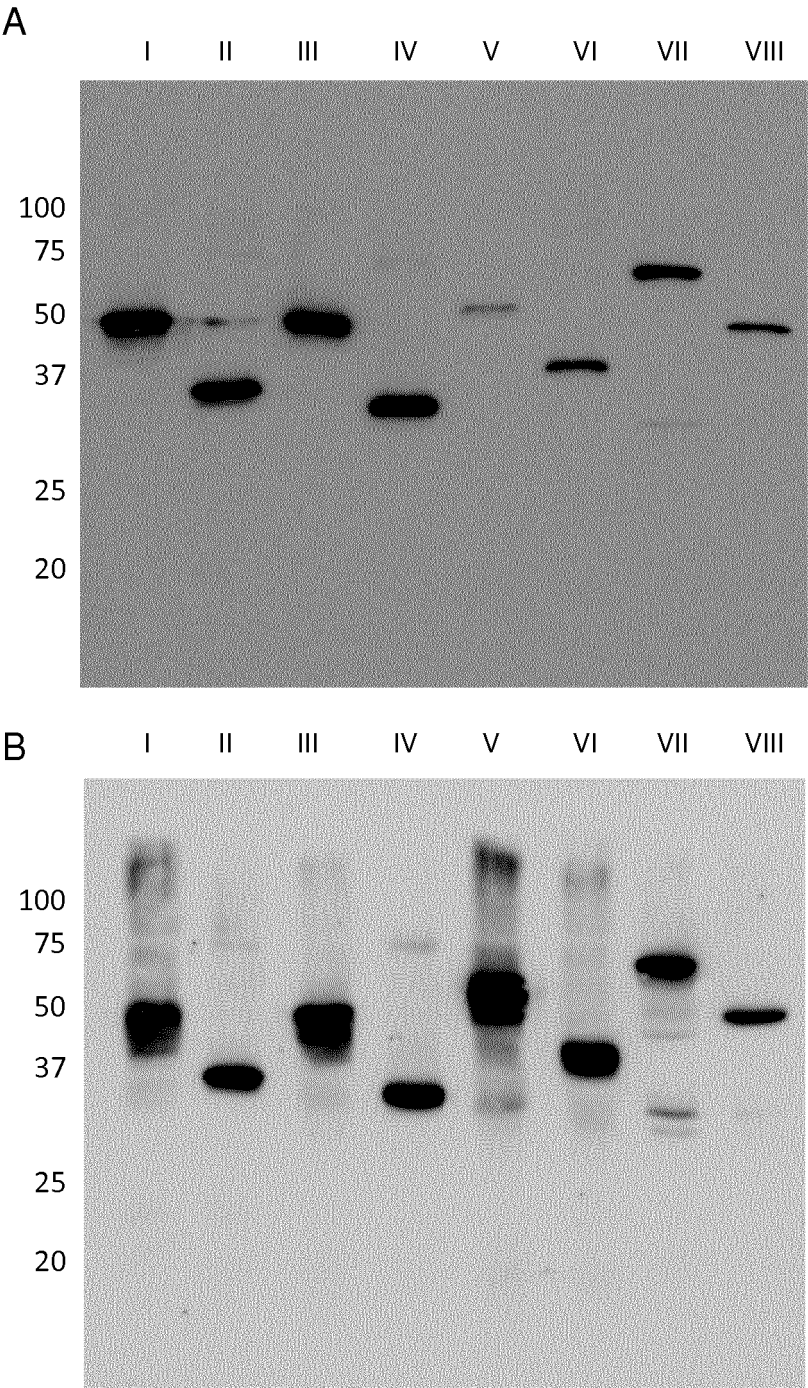


Figure 23

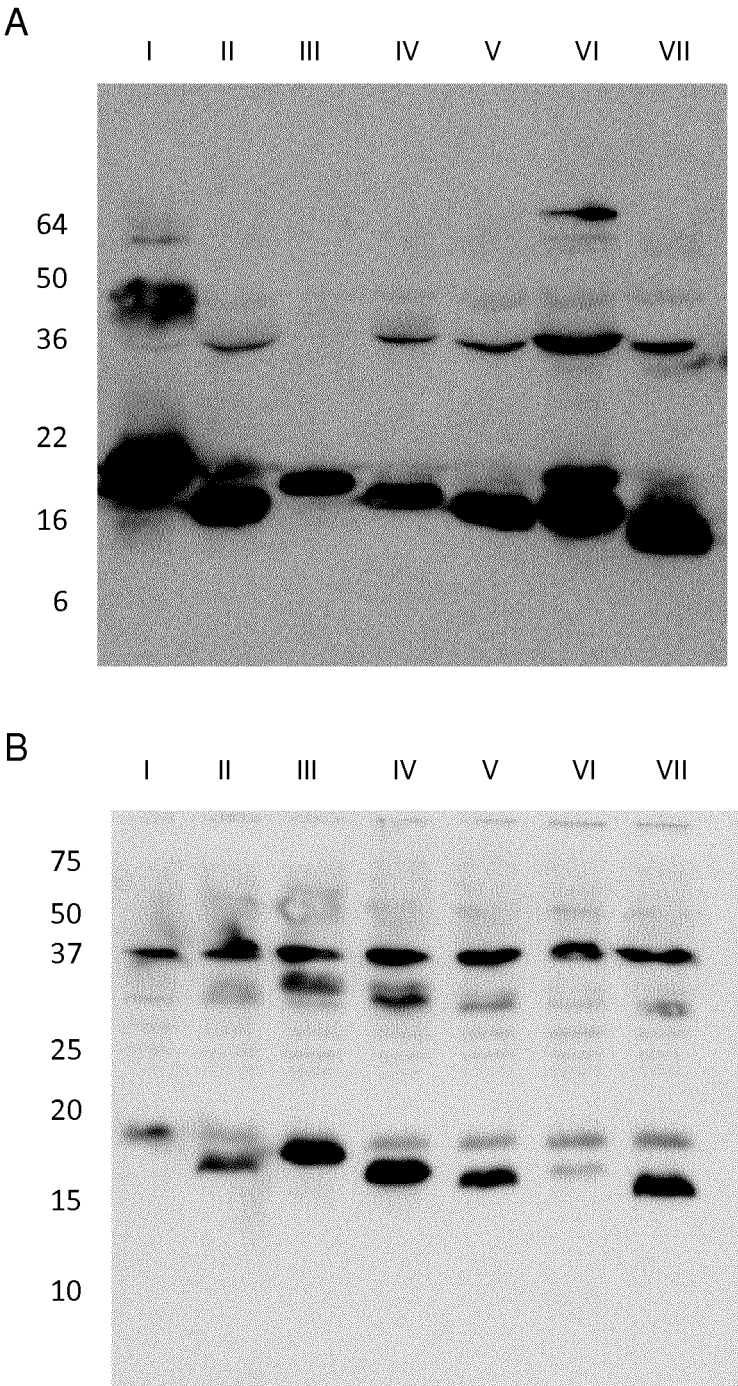


Figure 24

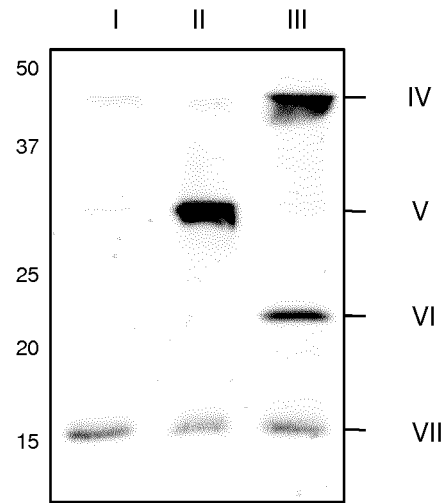


Figure 25

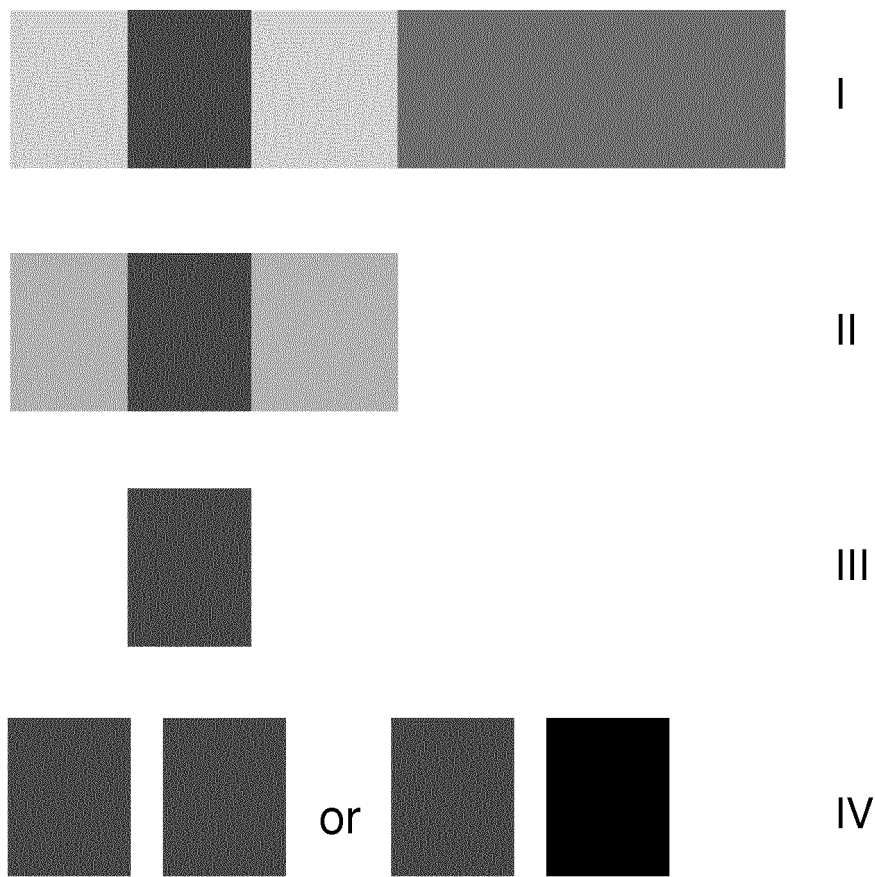


Figure 26

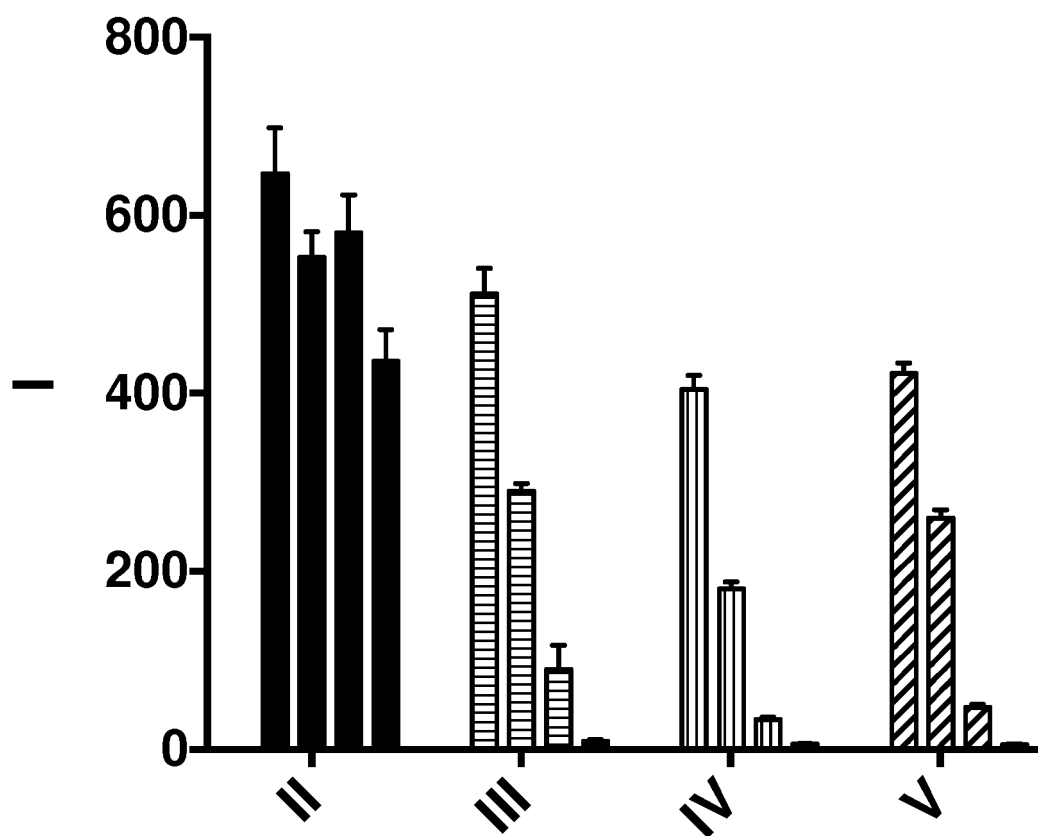


Figure 27

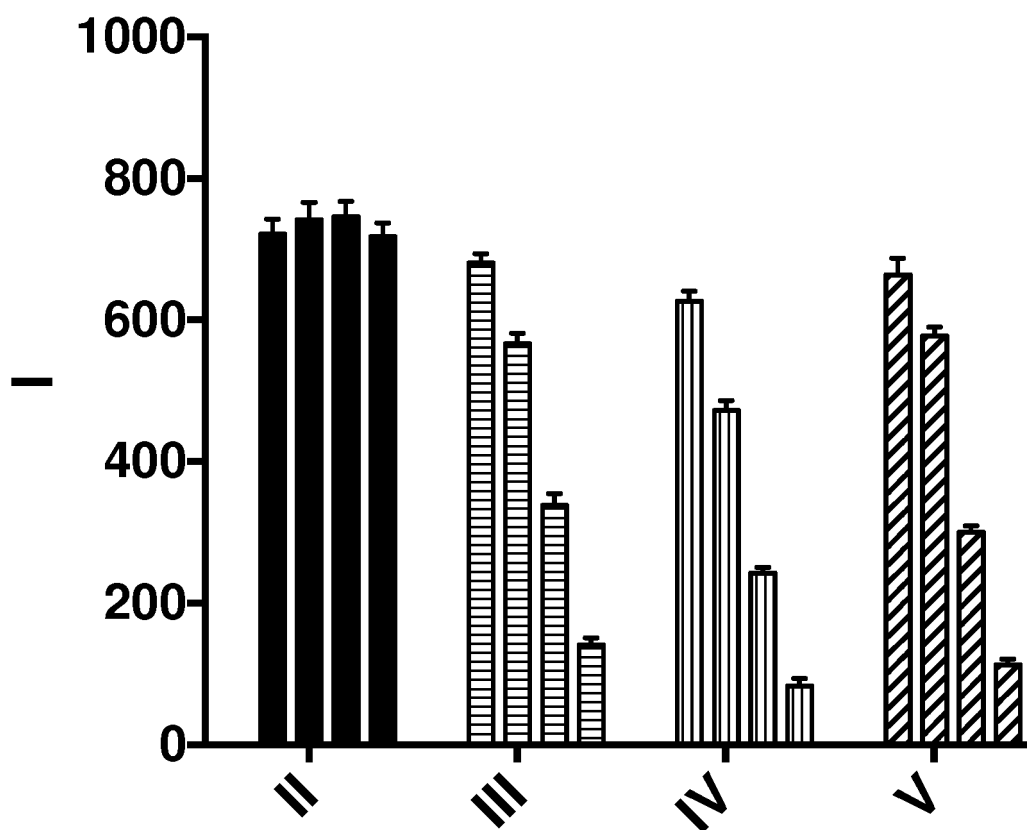


Figure 28

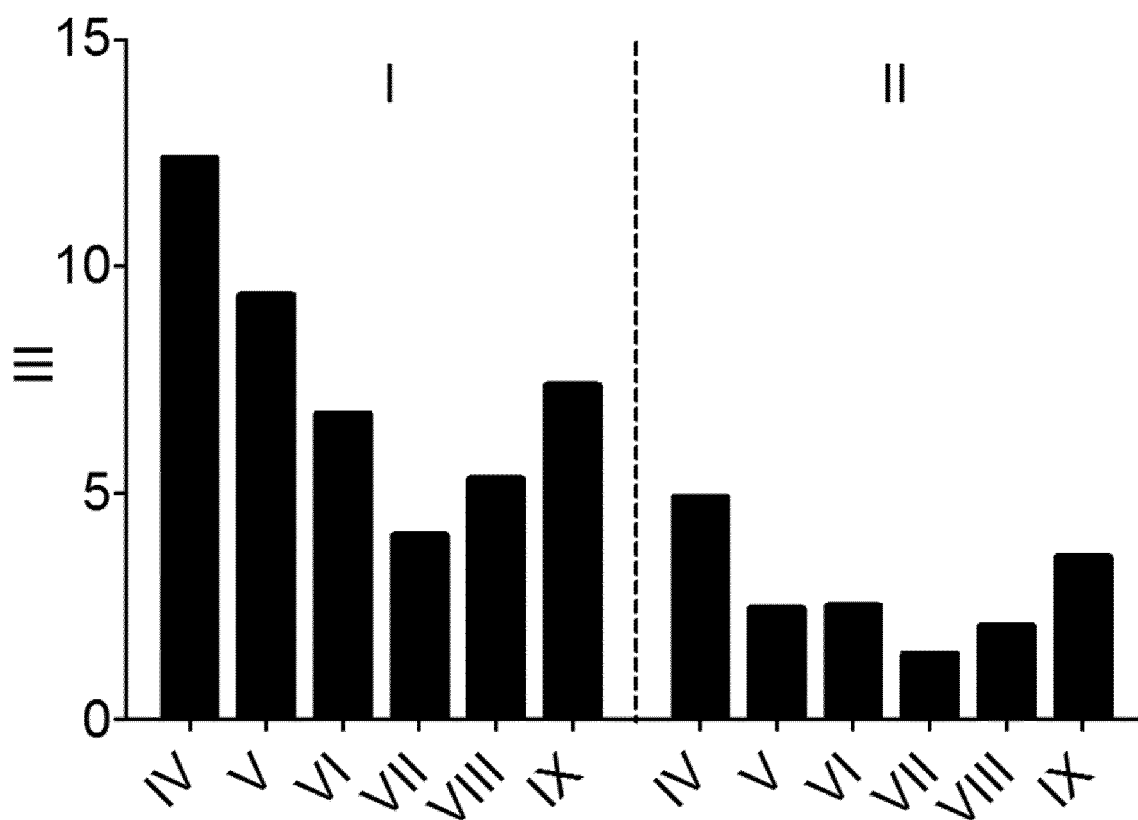


Figure 29

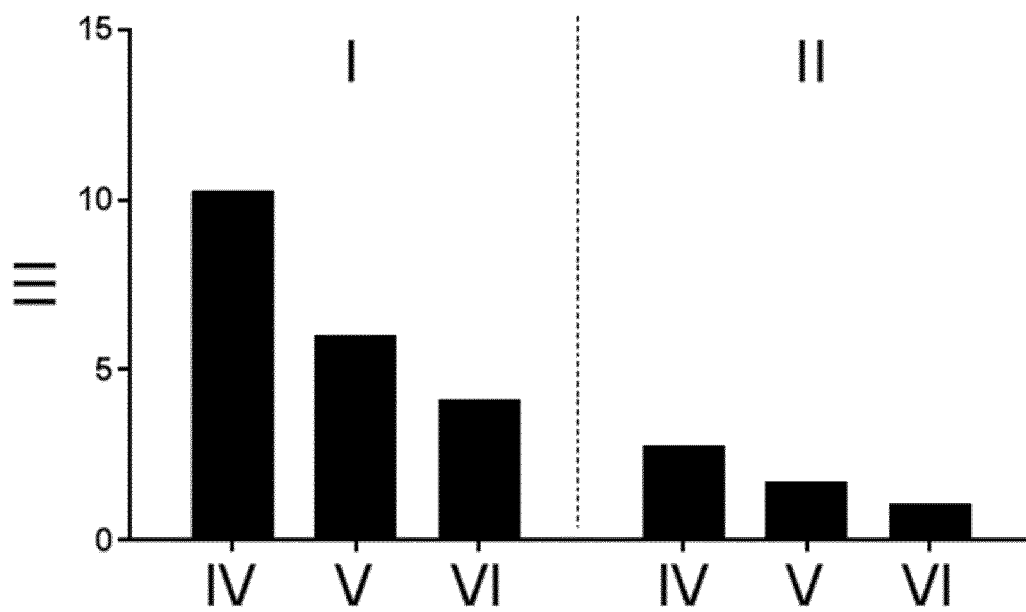


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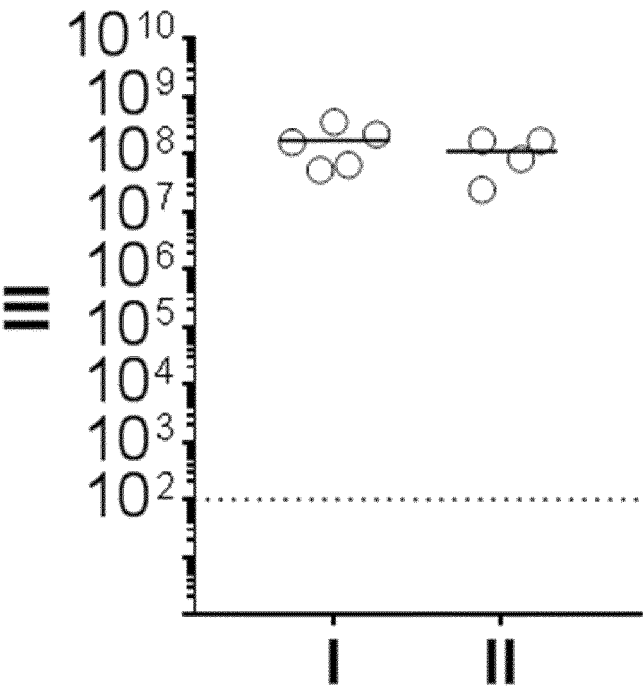


Figure 31

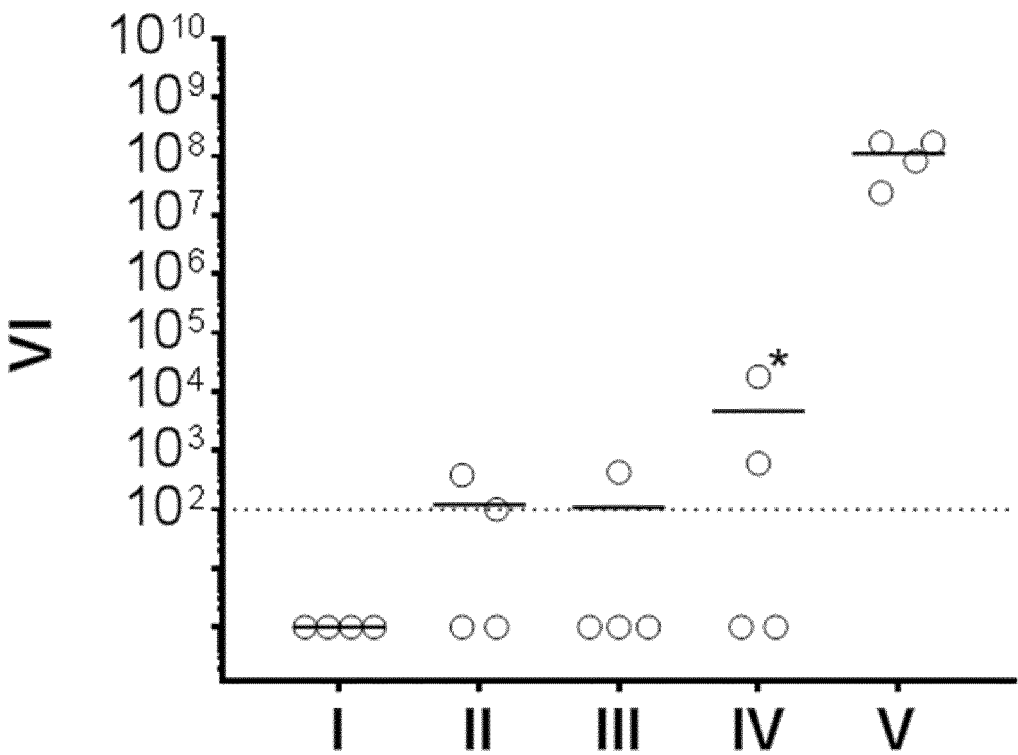


Figure 32

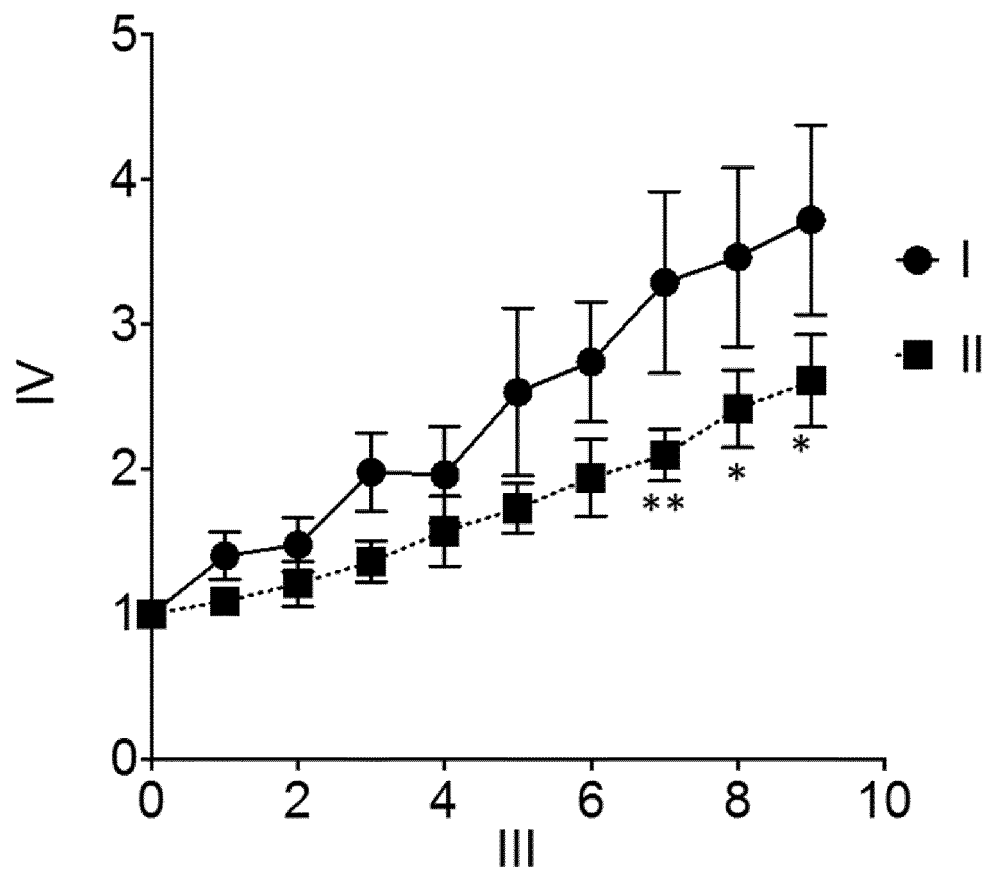


Figure 33

