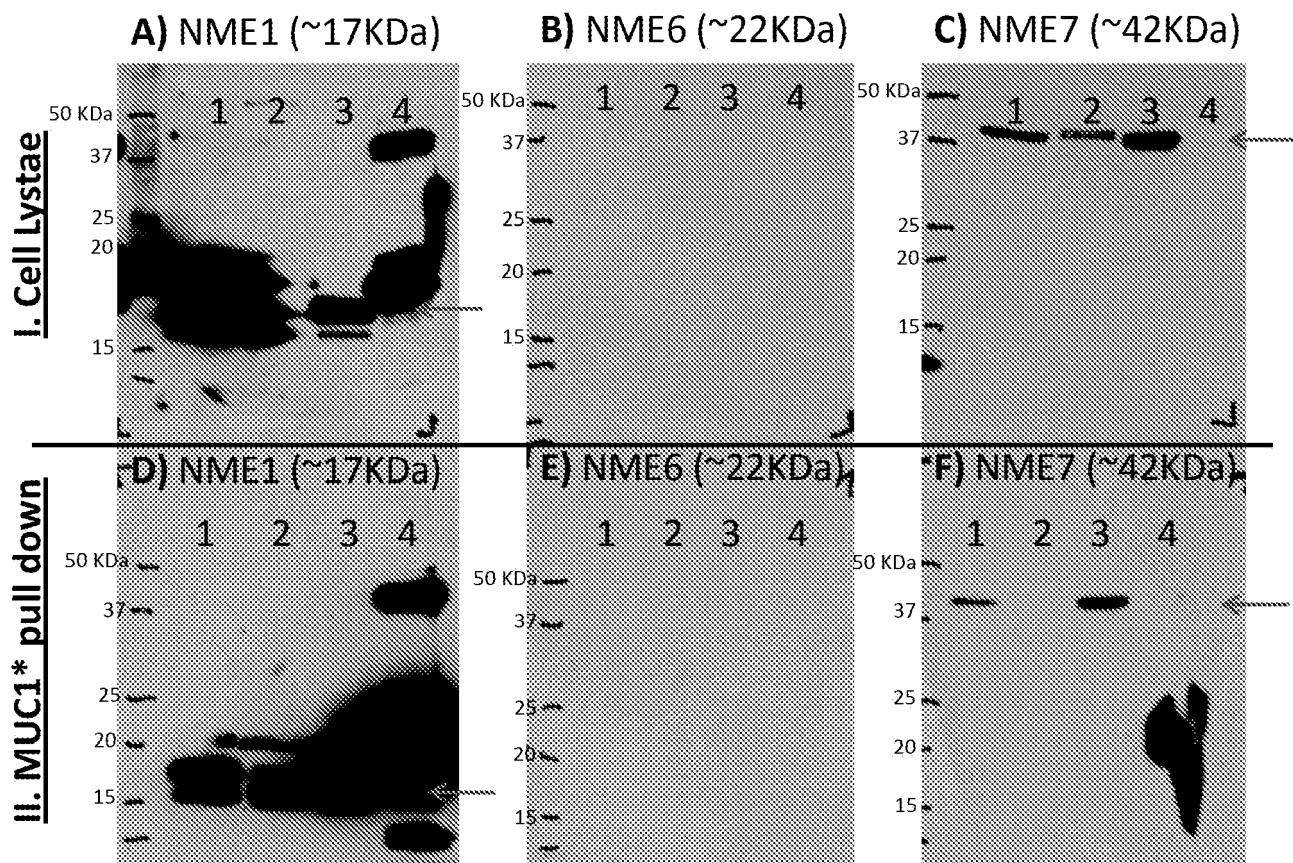




1- BGO1V vita plate/NM23
2- BGO1V MEF/FGF
3- T47D
4- NM23 WT

Fig. 1

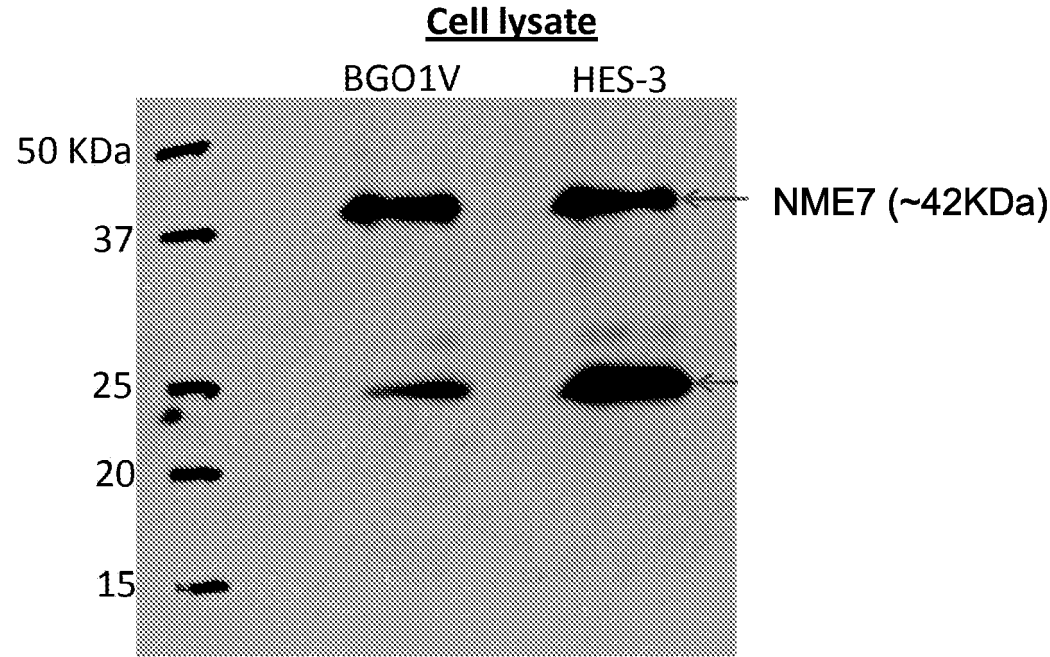


Fig. 2

3/30

NME1- S120G (purified dimers only): Day 4 : 4X Magnification

2.6 nM

8 nM

16 nM

26 nM

52 nM

104 nM

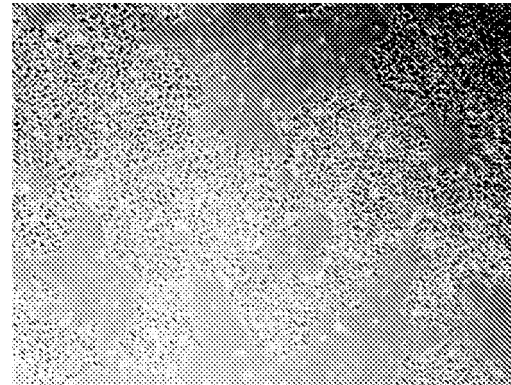
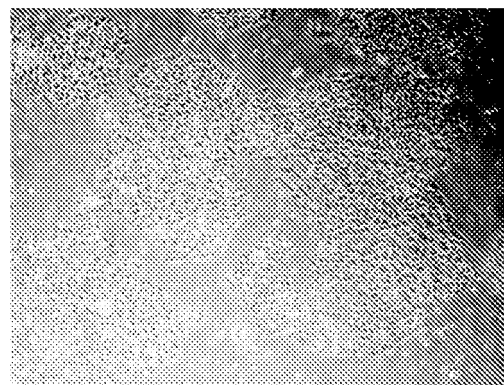
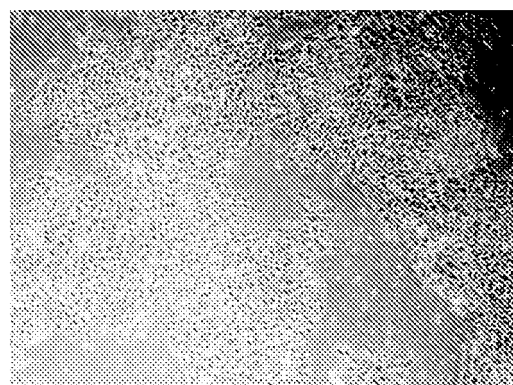
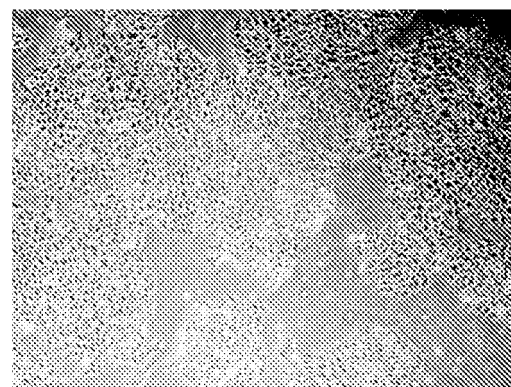
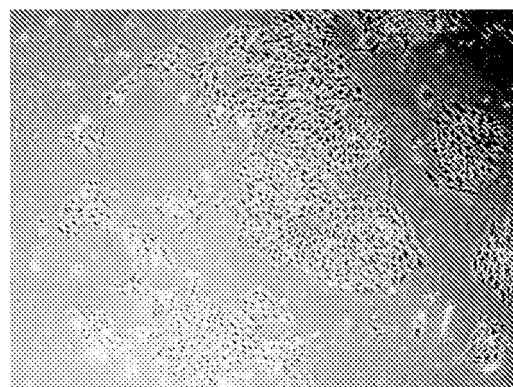
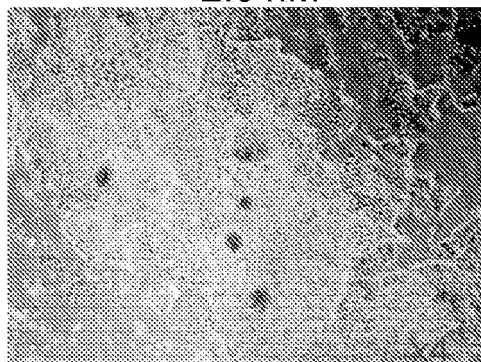


Fig. 3

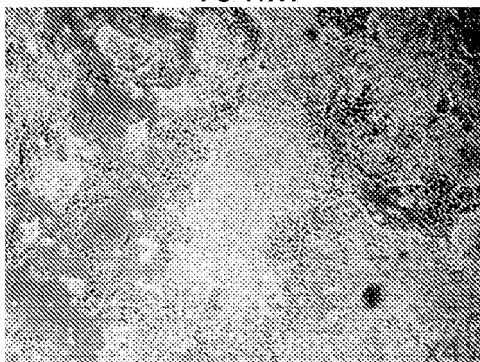
4/30

NME1- WT only: Day 4 : 4X Magnification

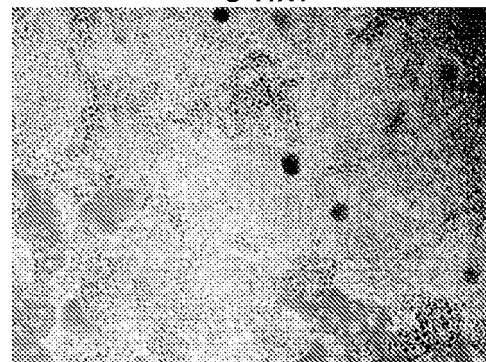
2.6 nM



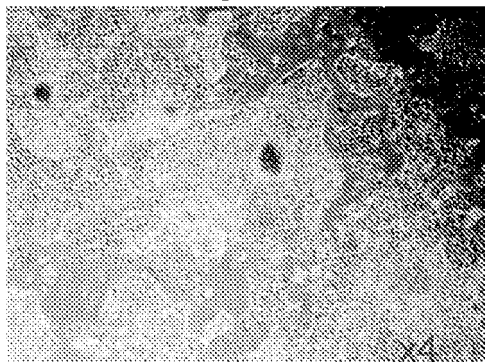
16 nM



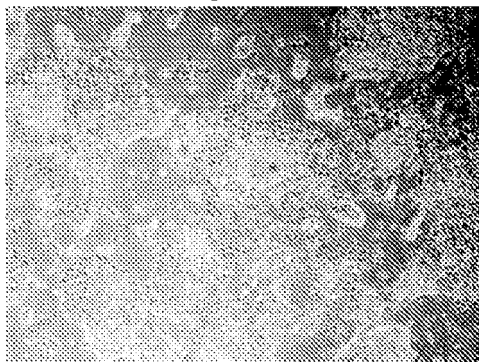
8 nM



26 nM



52 nM



104 nM

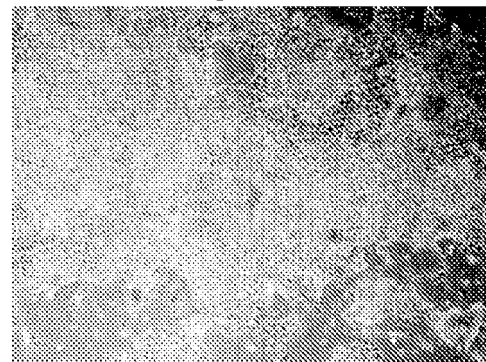
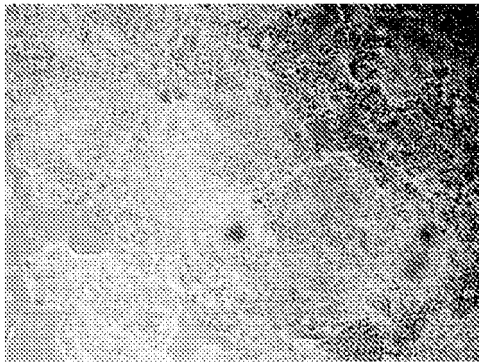


Fig. 4

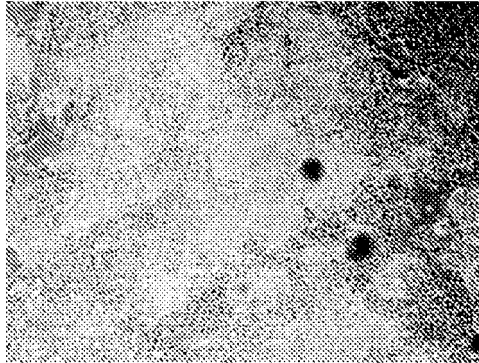
5/30

80% NME1 WT + 20% NME1-S120G Dimer : Day 4 4X Magnification

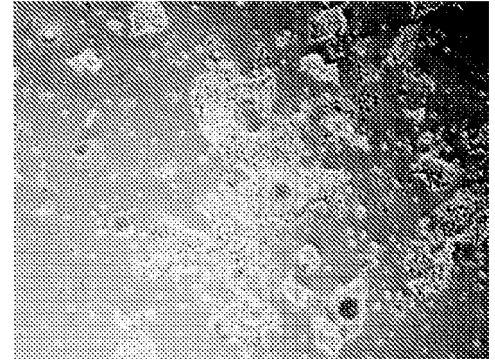
2.08 nM wt hexamer + 0.52 nM dimer
2.6nM total



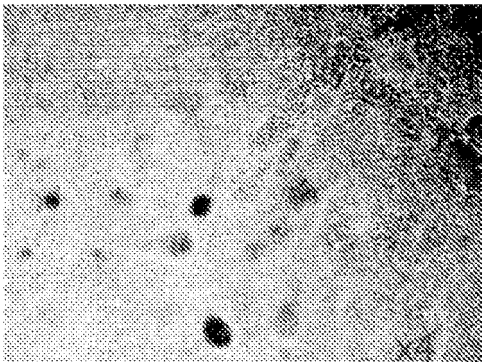
6.4 nM wt hexamer + 1.6 nM dimer
8nM total



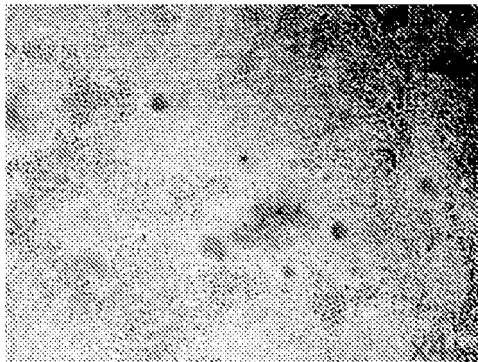
12.8 nM wt hexamer + 3.2 nM dimer
16nM total



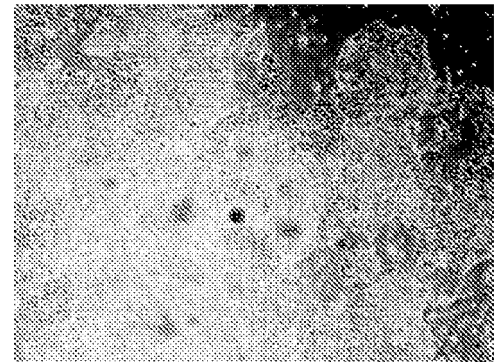
20.8 nM wt hexamer + 5.2 nM dimer
26nM total



41.6 nM wt hexamer + 10.4 nM dimer
52nM total



83.2 nM wt hexamer + 20.8 nM dimer
104nM total

**Fig. 5**

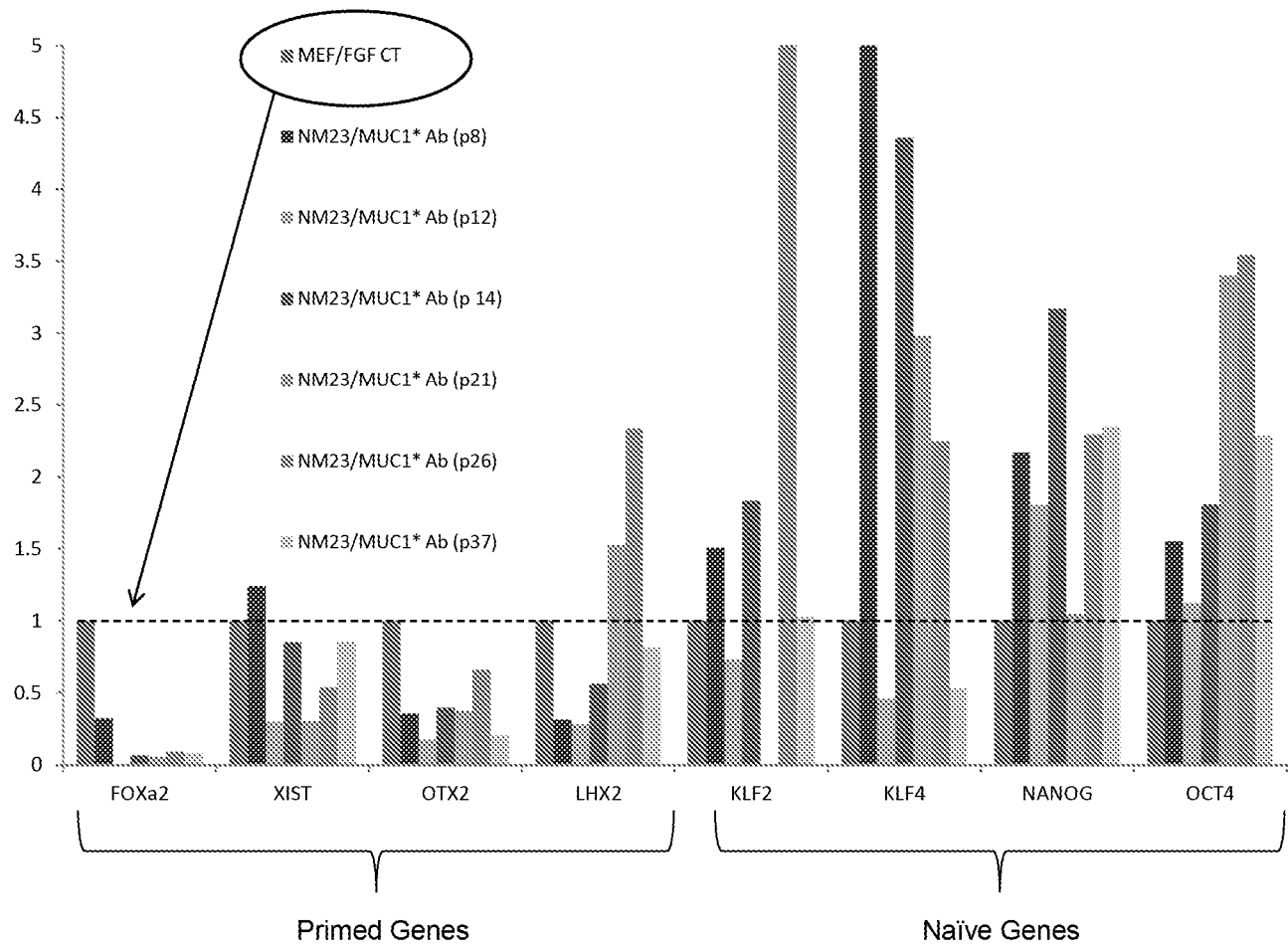
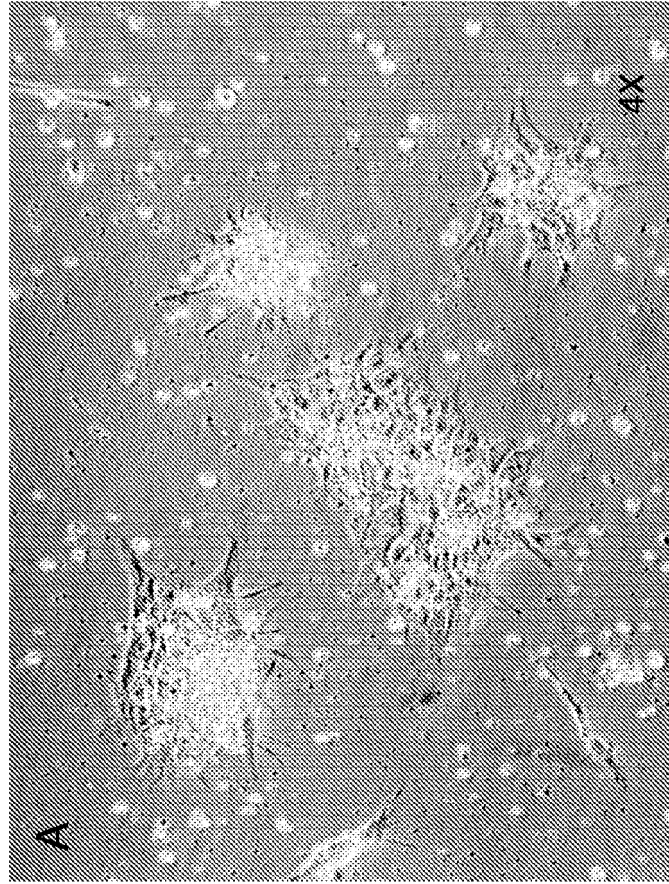


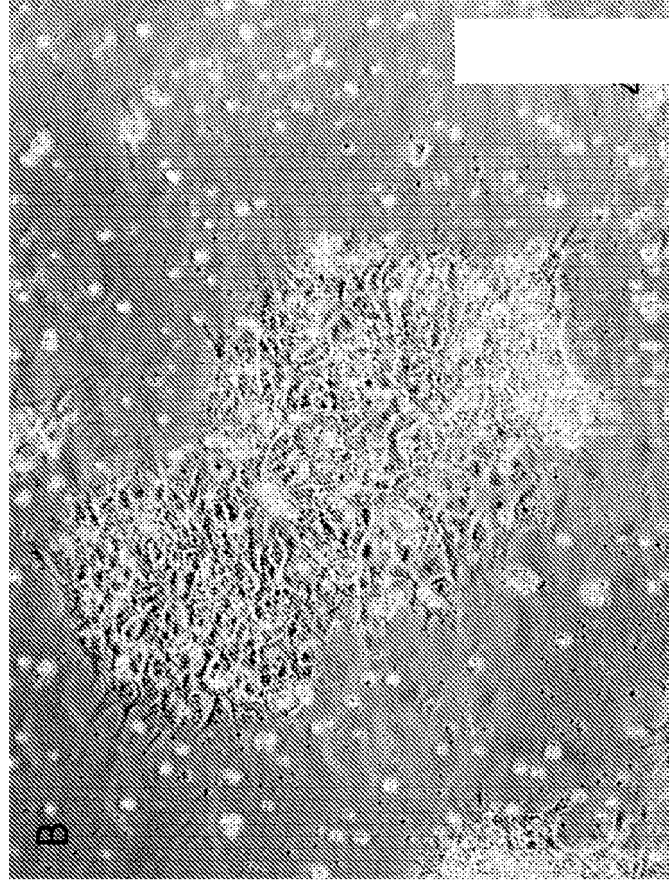
Fig. 6

Suppression of NM23 – NME1, NME6, and NME7 results in loss of growth and induction of differentiation



siRNA knocks down NME1, NME6, & NME7

Cells are sparse and differentiating at 48 hours post plating.
No growth factor added into serum-free, minimal stem cell growth media.



Negative Control: 3X scrambled siRNA added

More cells and not differentiating at 48 hours post plating
Recombinant NME1, purified as stable dimers added in serum-free, minimal stem cell growth media.

Fig. 7

Suppression of NME7 is worst single NME knockdown; Suppression of NME1 has little effect;

WO 2014/018679

8/30

PCT/US2013/051899

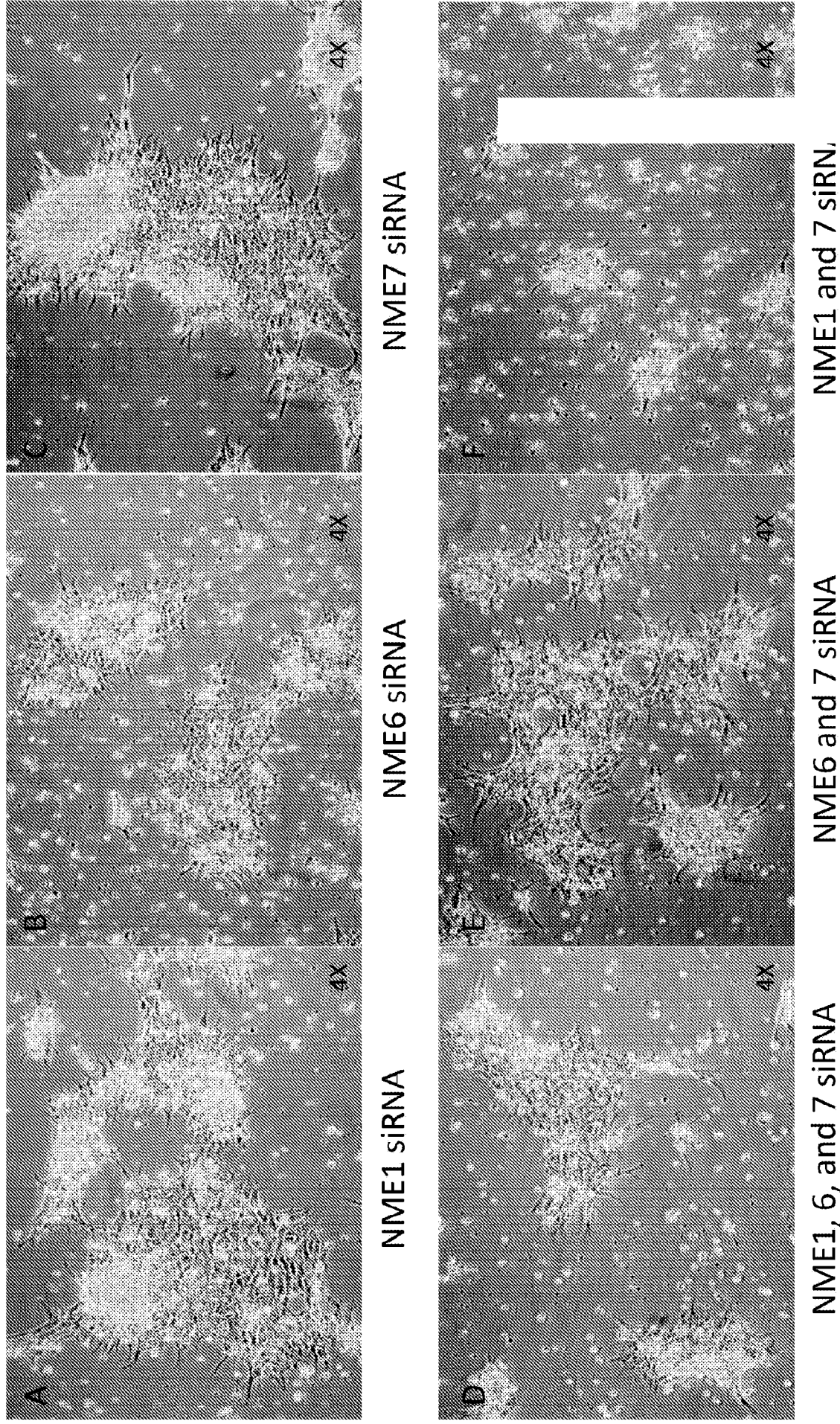


Fig. 8

Differentiation induced by Suppression of NME7 is prevented by adding recombinant NME1, refolded and purified as dimer

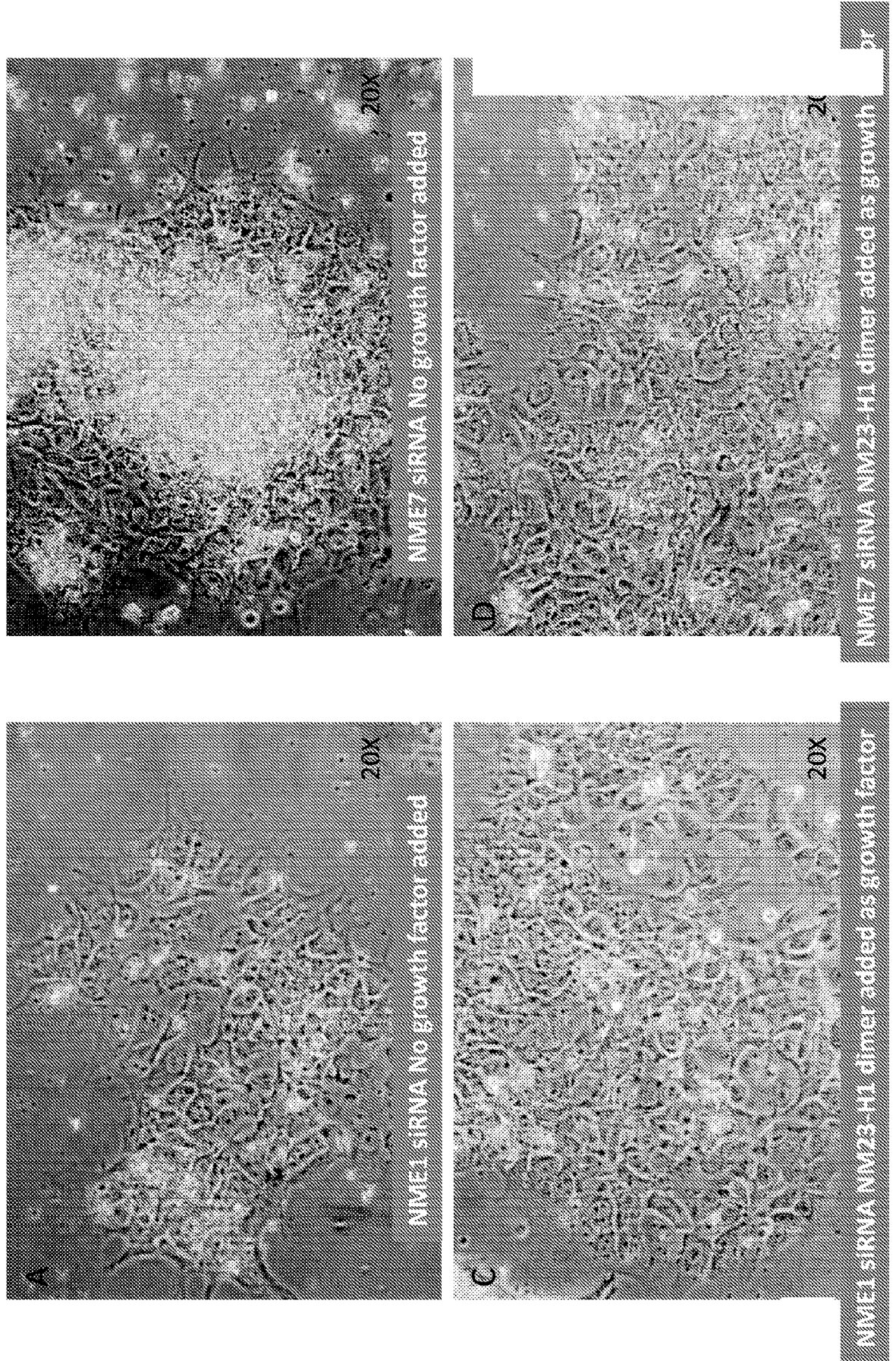
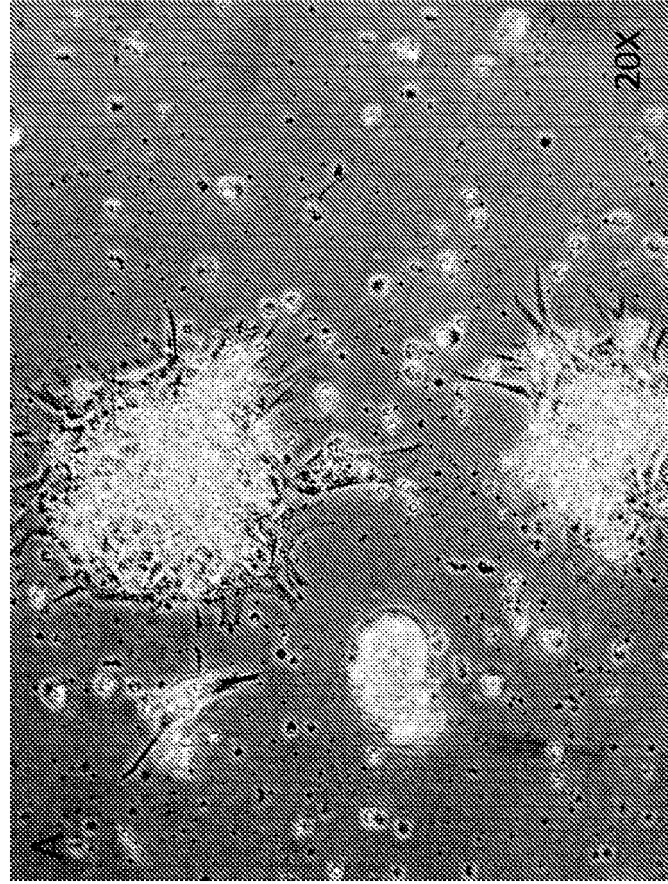
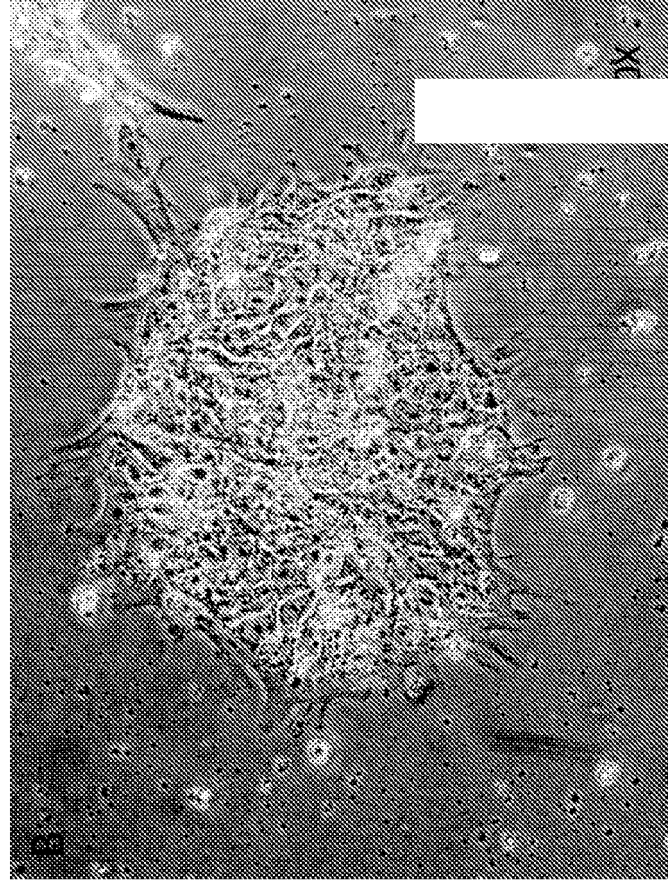


Fig. 9

Differentiation induced by Suppression of NME1, NME6 and NME7 is prevented by adding recombinant NME1, refolded and purified as dimer



siRNA knocks down NME1, NME6, & NME7
Cells are sparse and differentiating at 48 hours post plating.
No growth factor added into serum-free, minimal stem cell growth media.



siRNA knocks down NME1, NME6, & NME7. Recombinant NME1 refolded and purified as stable dimer is added into serum-free, minimal stem cell growth media. Cell number is increased and undifferentiated.

Fig. 10

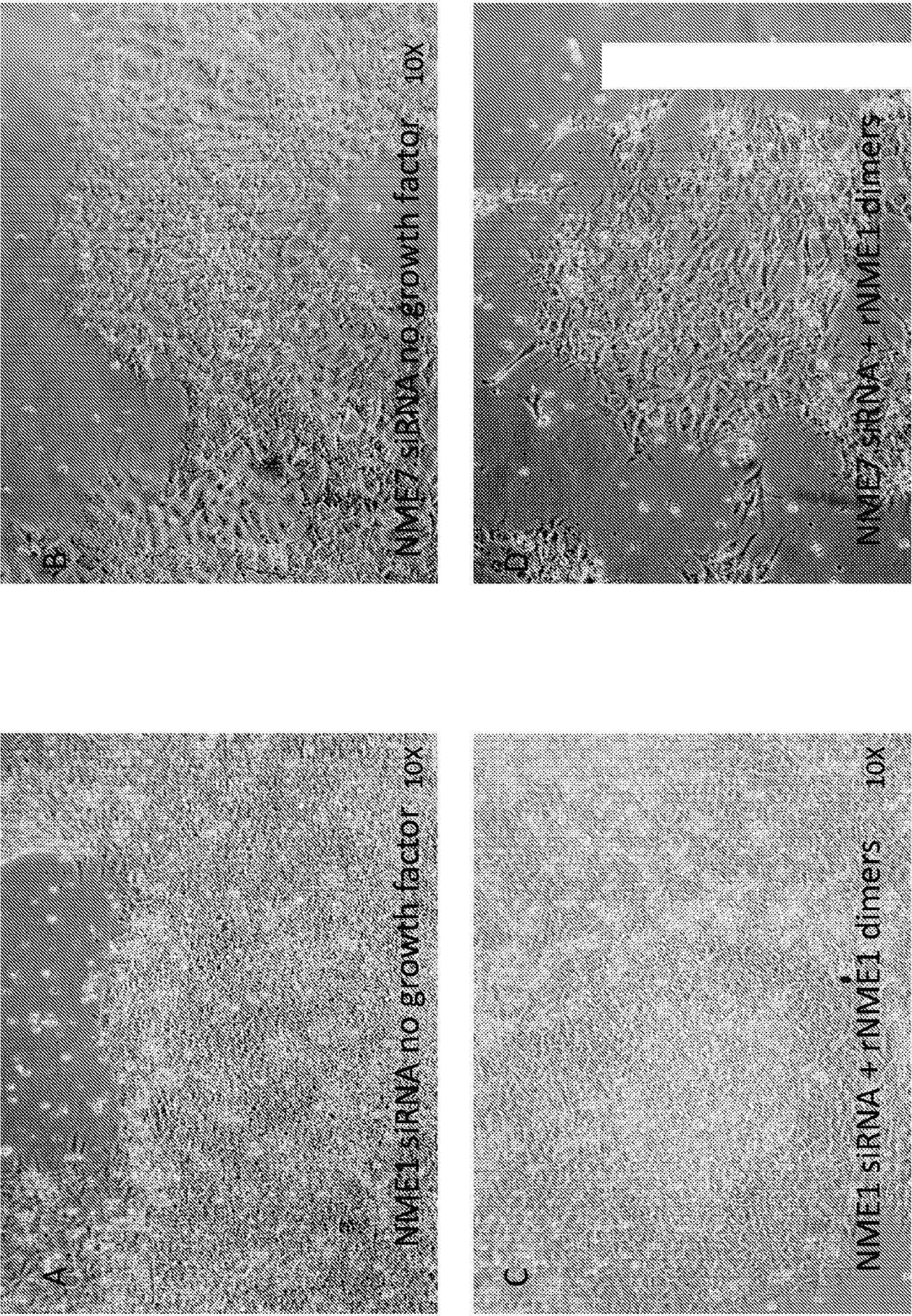


Fig. 11

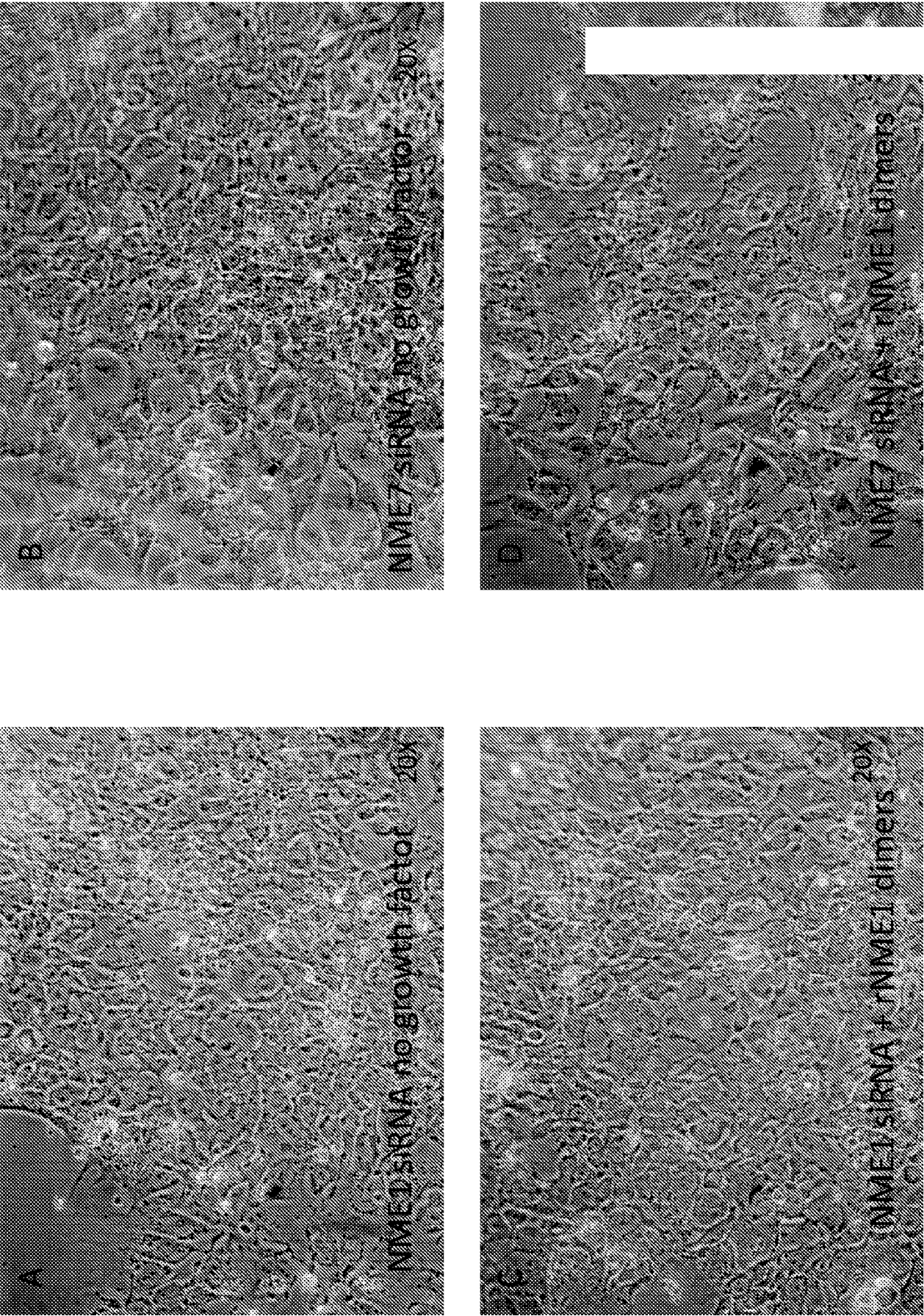


Fig. 12

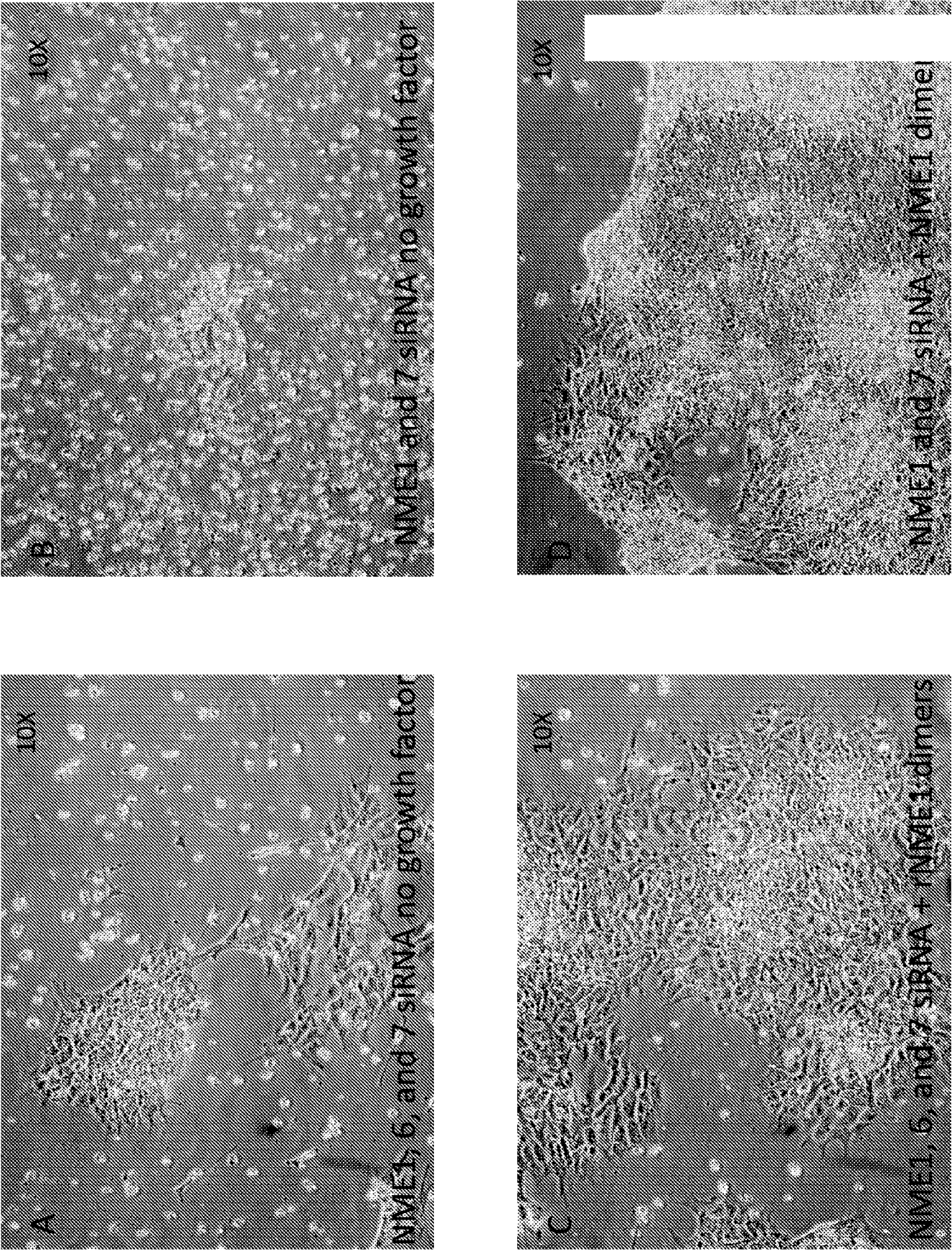


Fig. 13

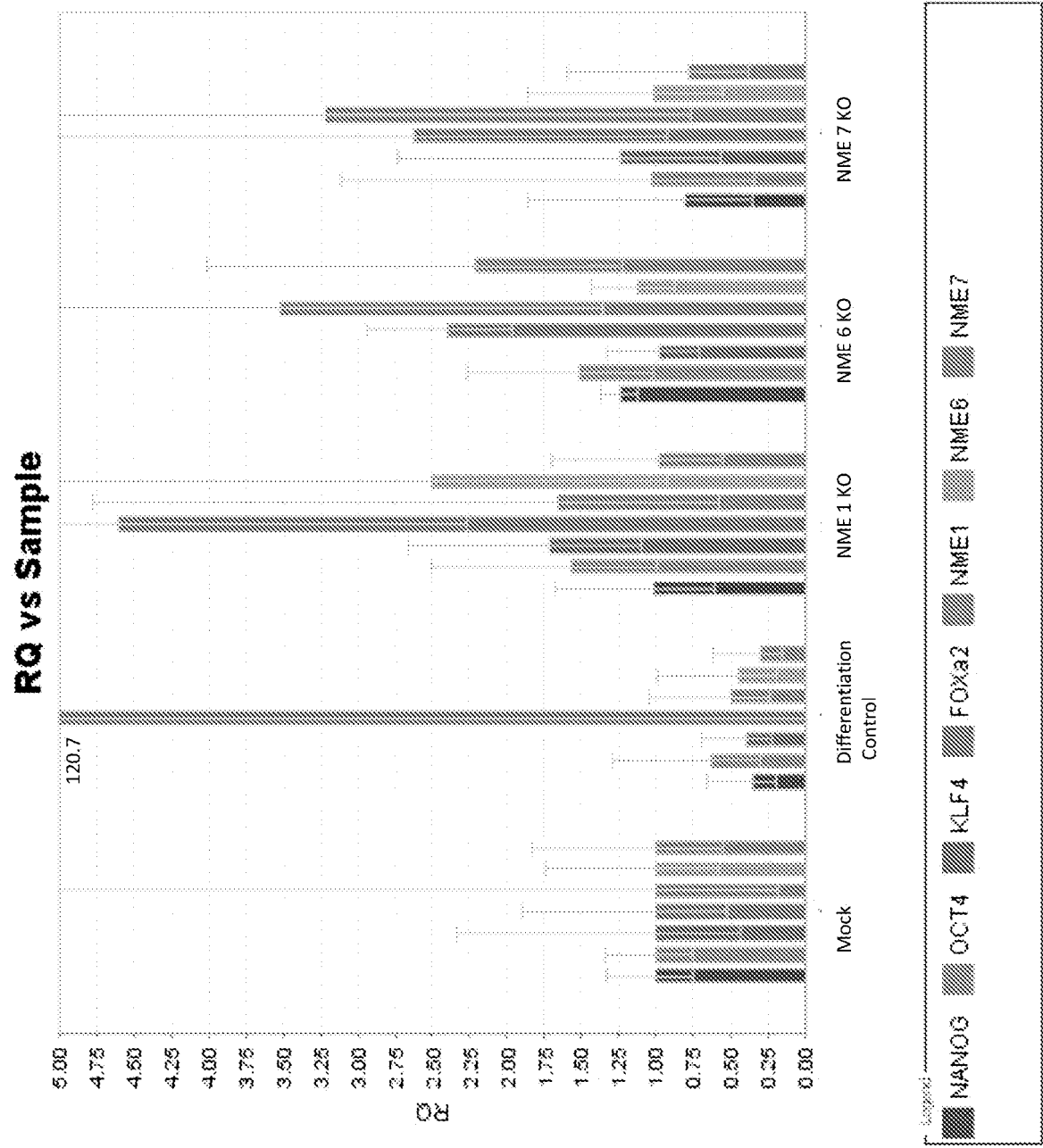


Fig. 14

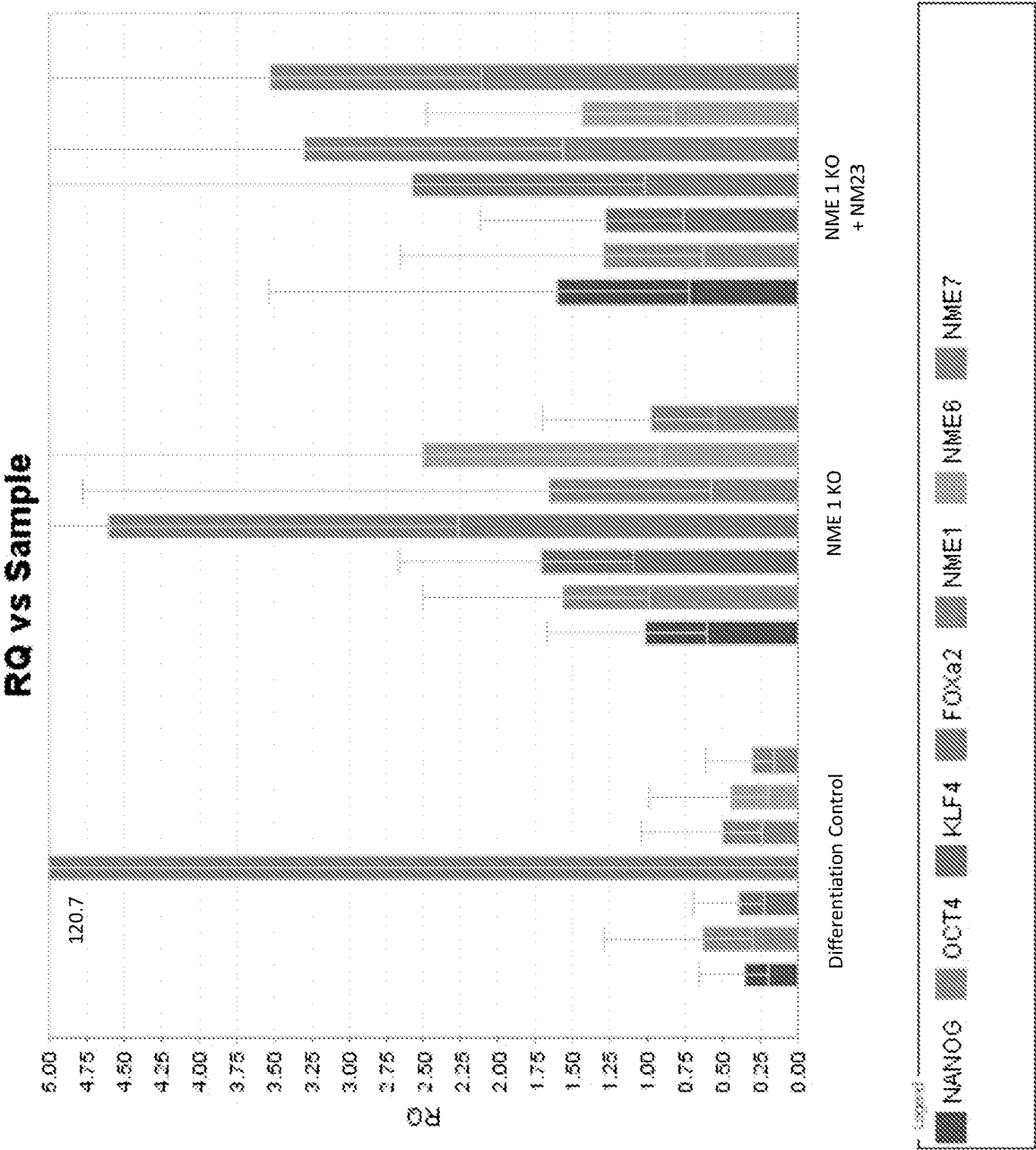


Fig. 15

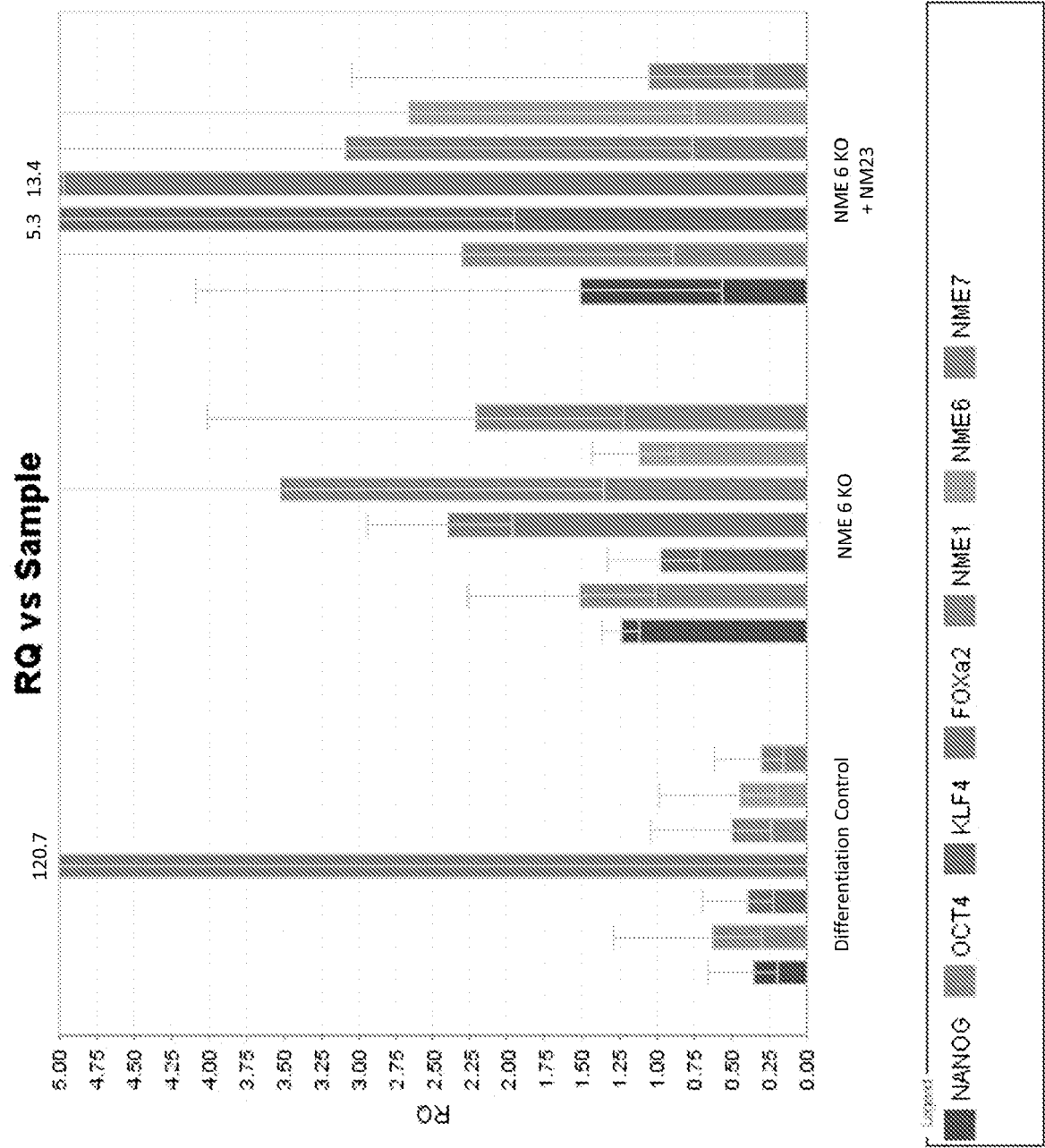


Fig. 16

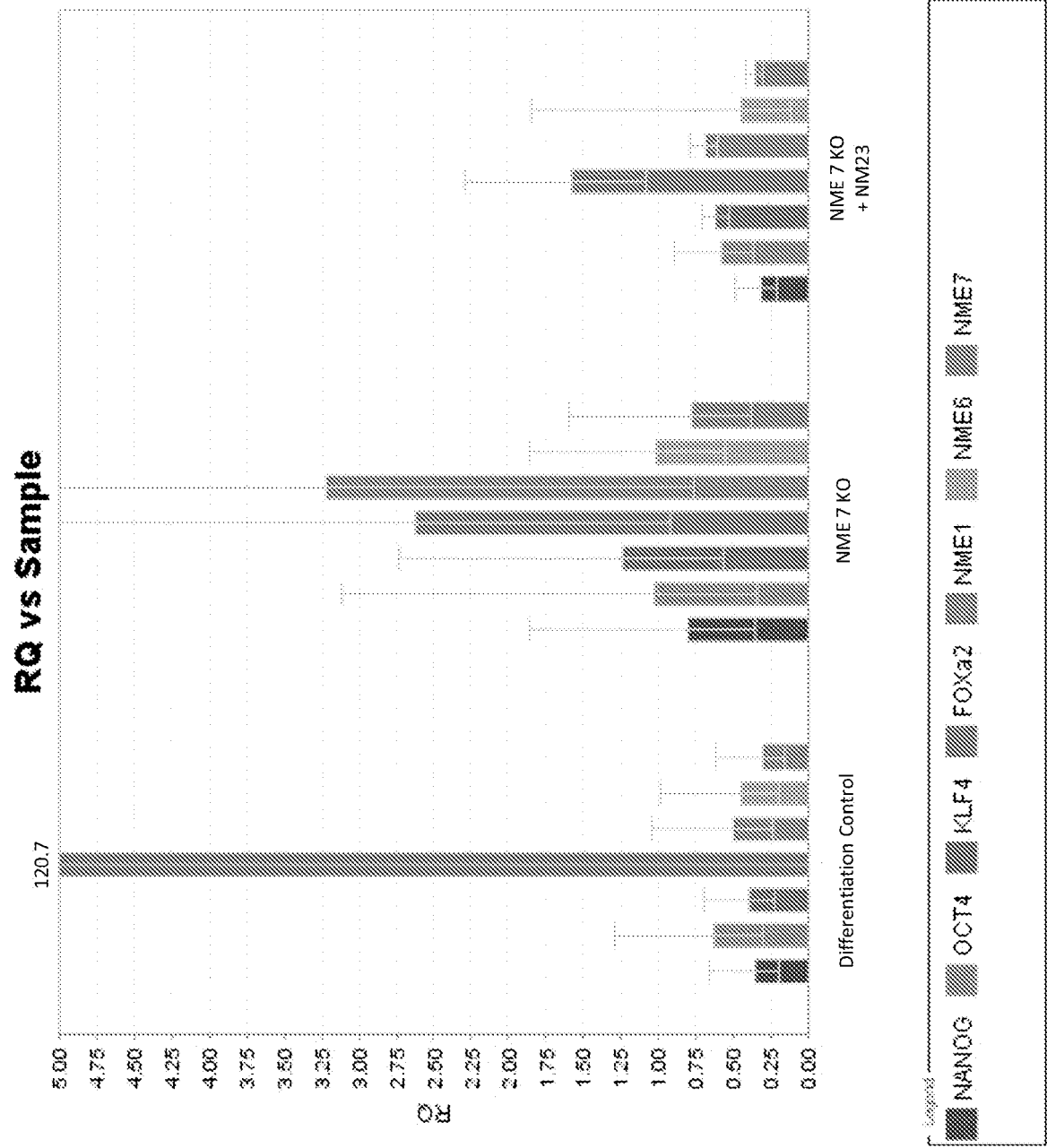


Fig. 17

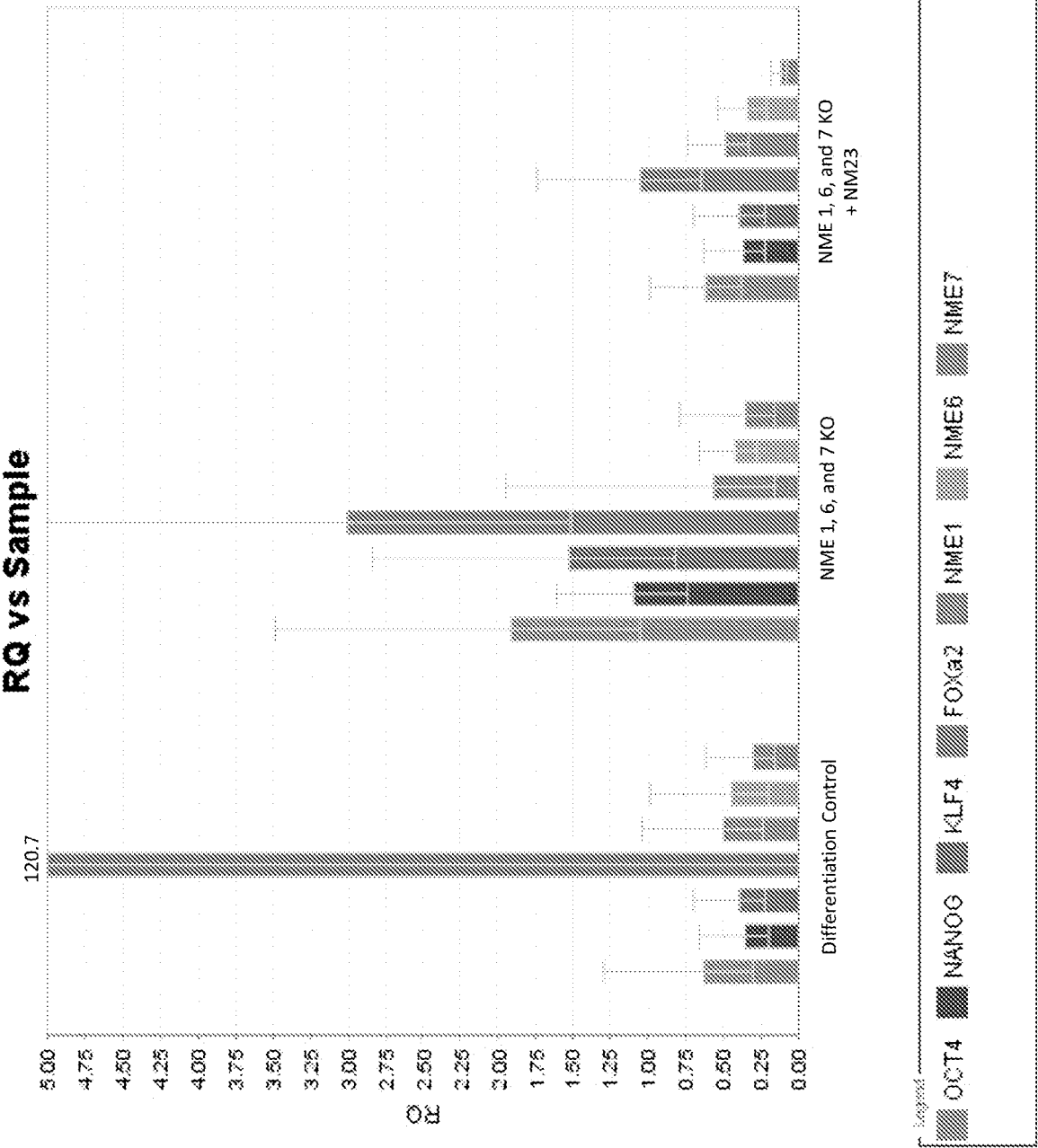


Fig. 18

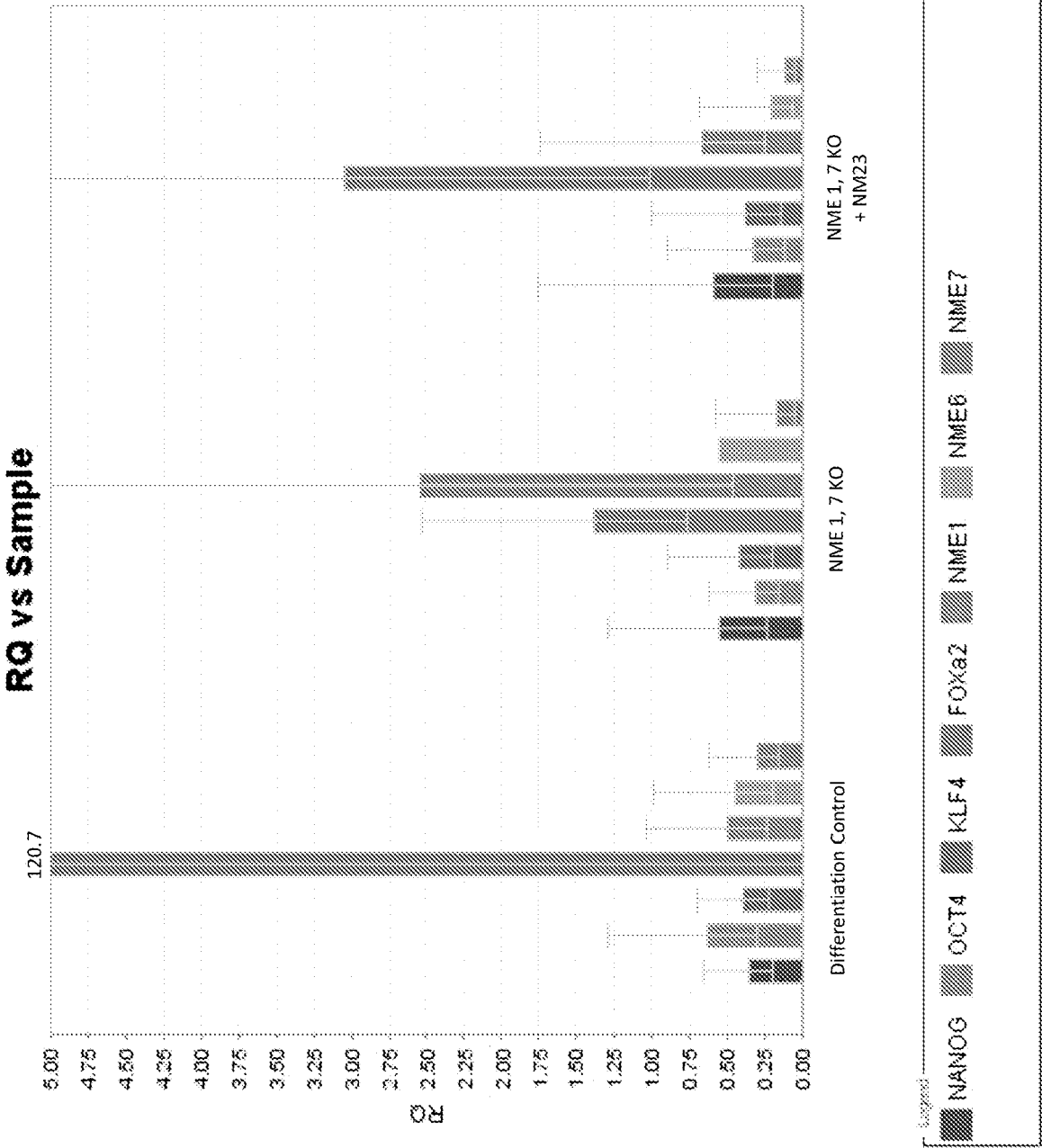
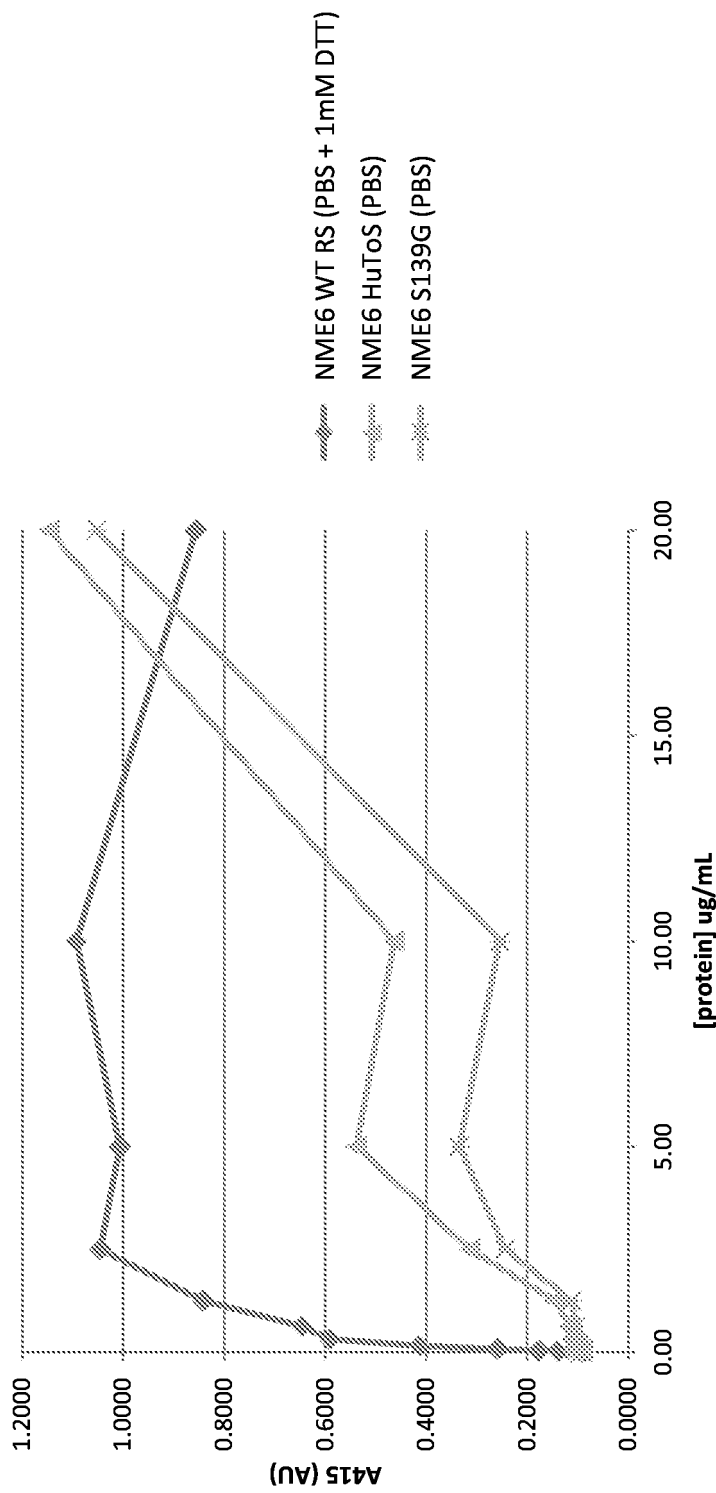
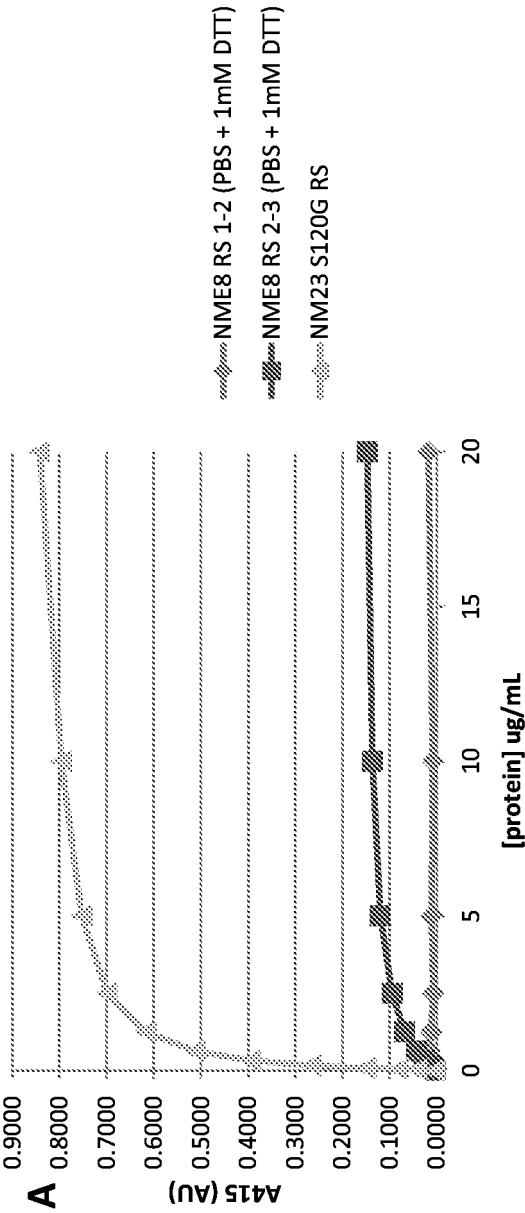


Fig. 19



-By FPLC:
-NME6 WT RS (PBS+1mM DTT) = monomer
-NME6 HuToS (PBS) = monomer
-NME6 S139G (PBS) = monomer

Figure 20



-By FPLC:
-NME8 RS 1-2 (PBS + 1mM DTT) = monomer
-NME8 RS 2-3 (PBS + 1mM DTT) = oligomer

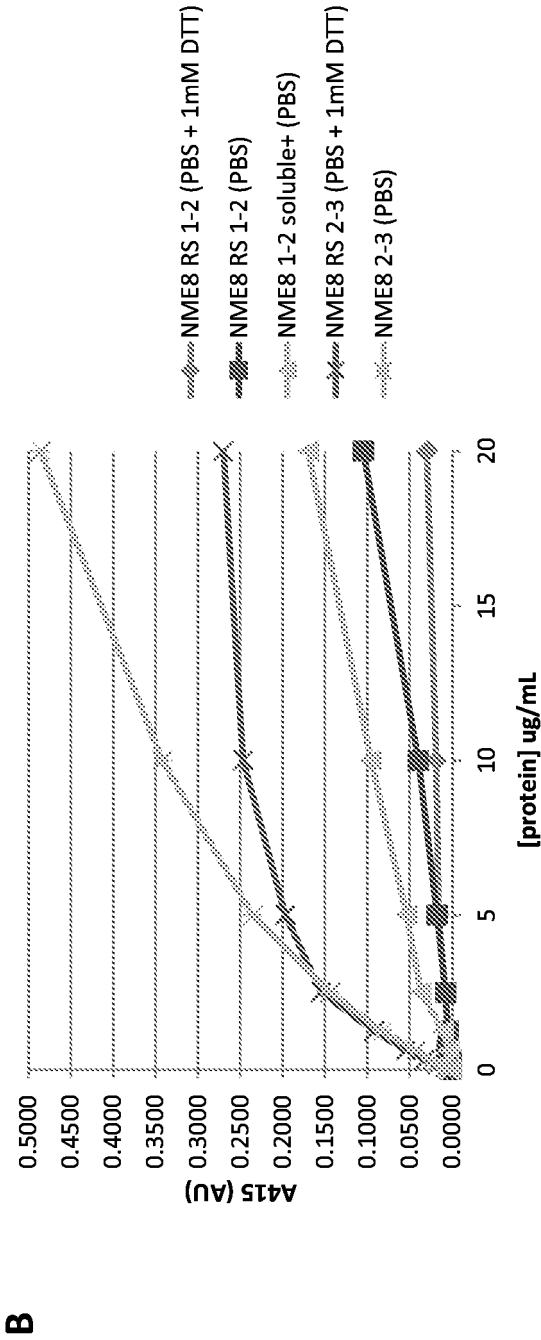


Figure 21

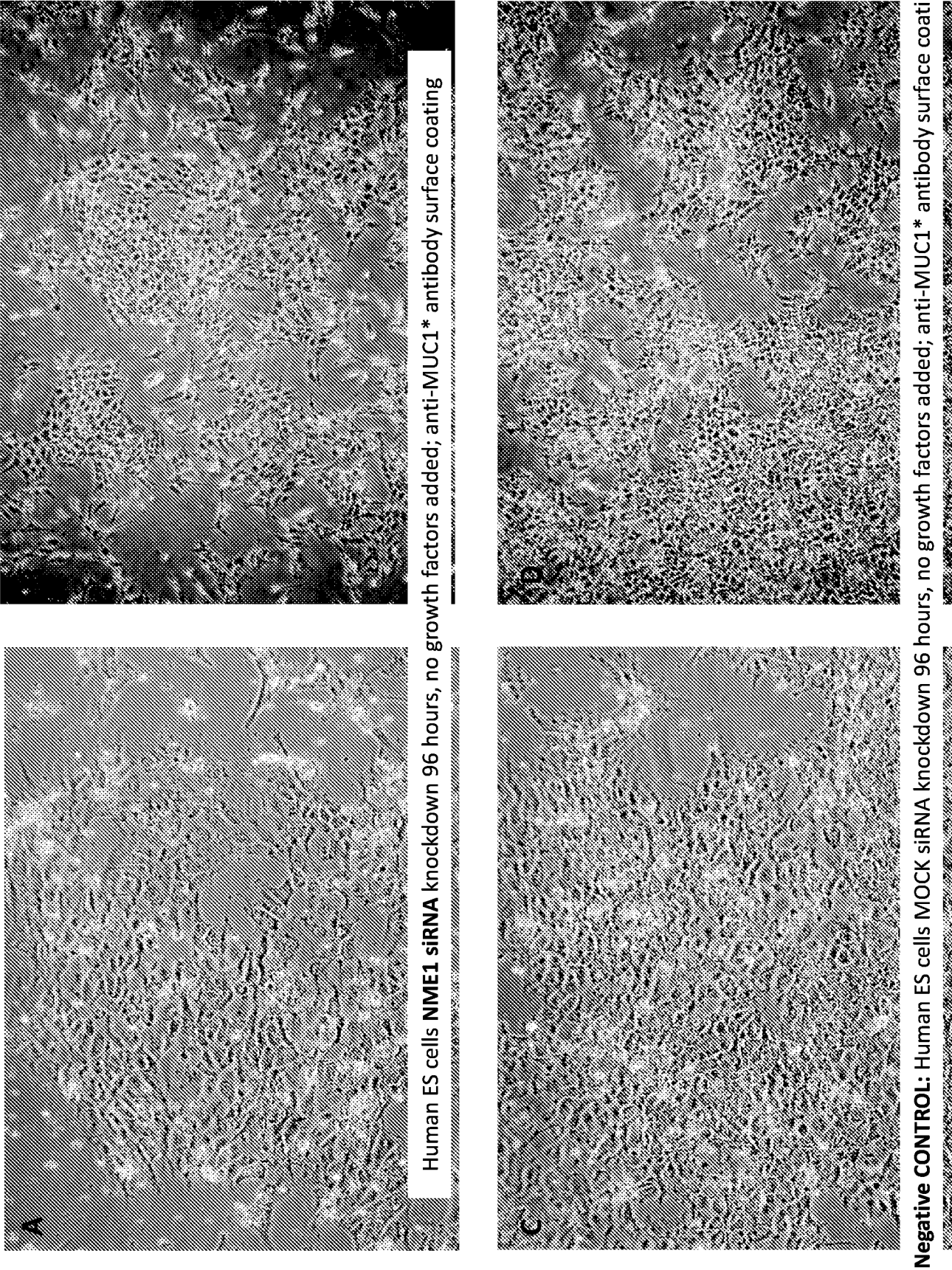
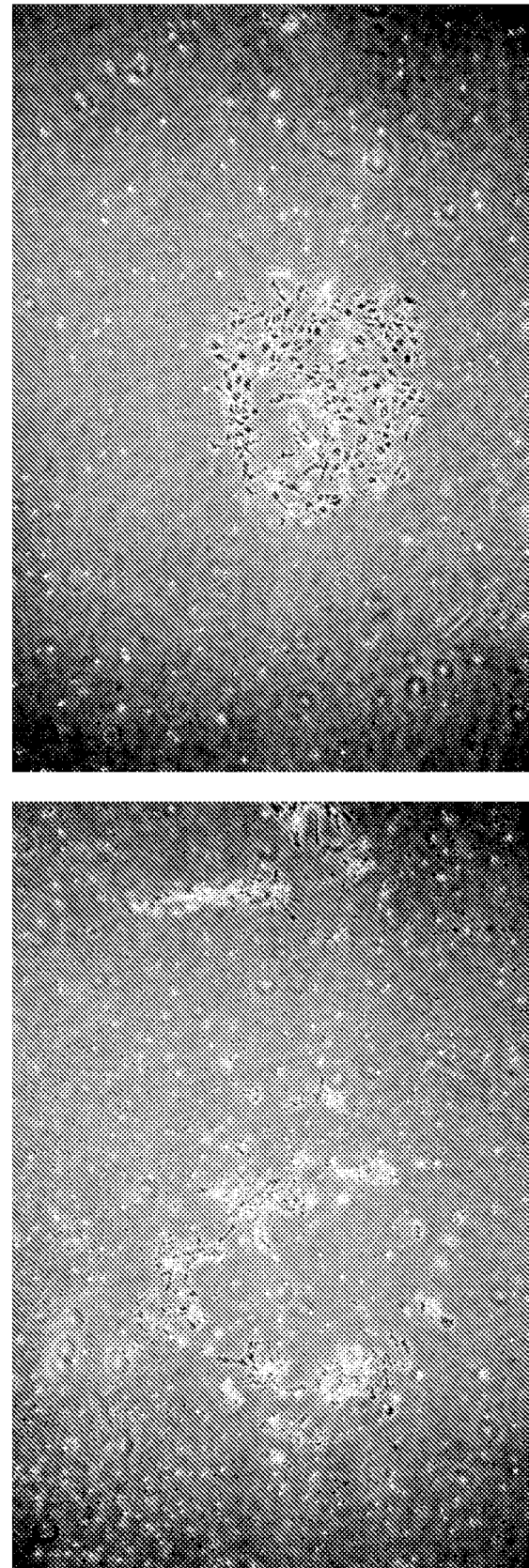
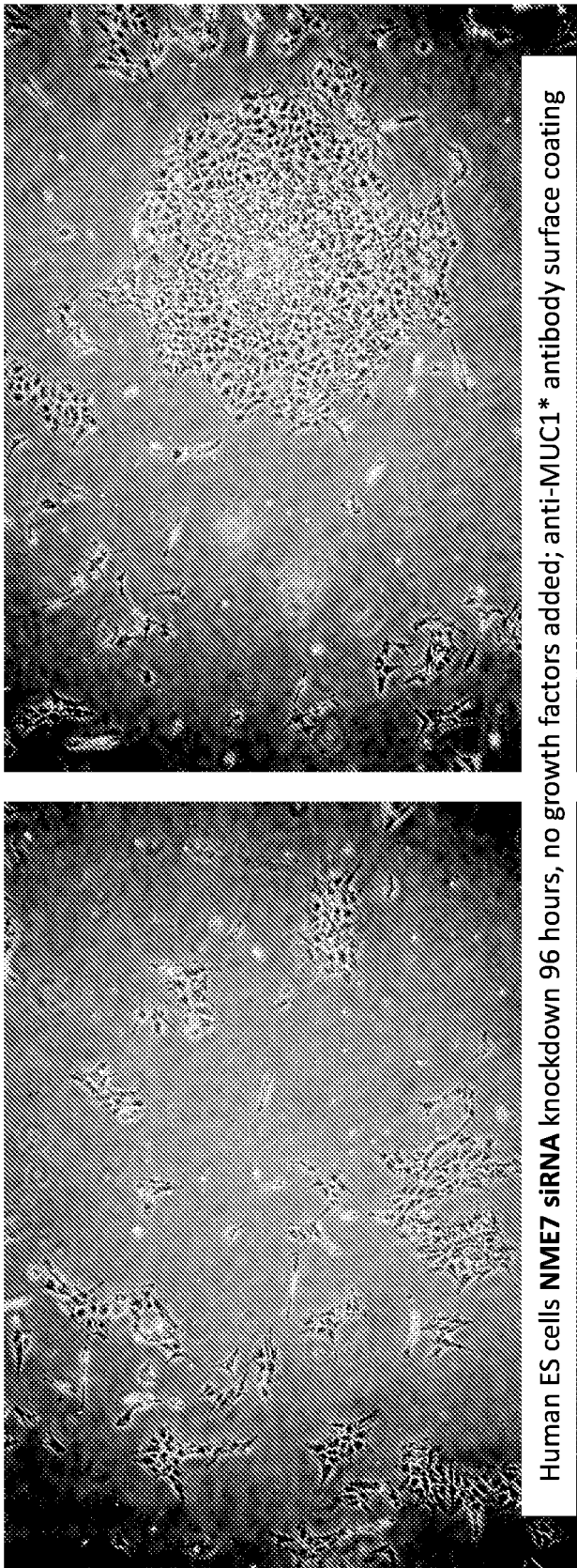


Figure 22

**Figure 23**

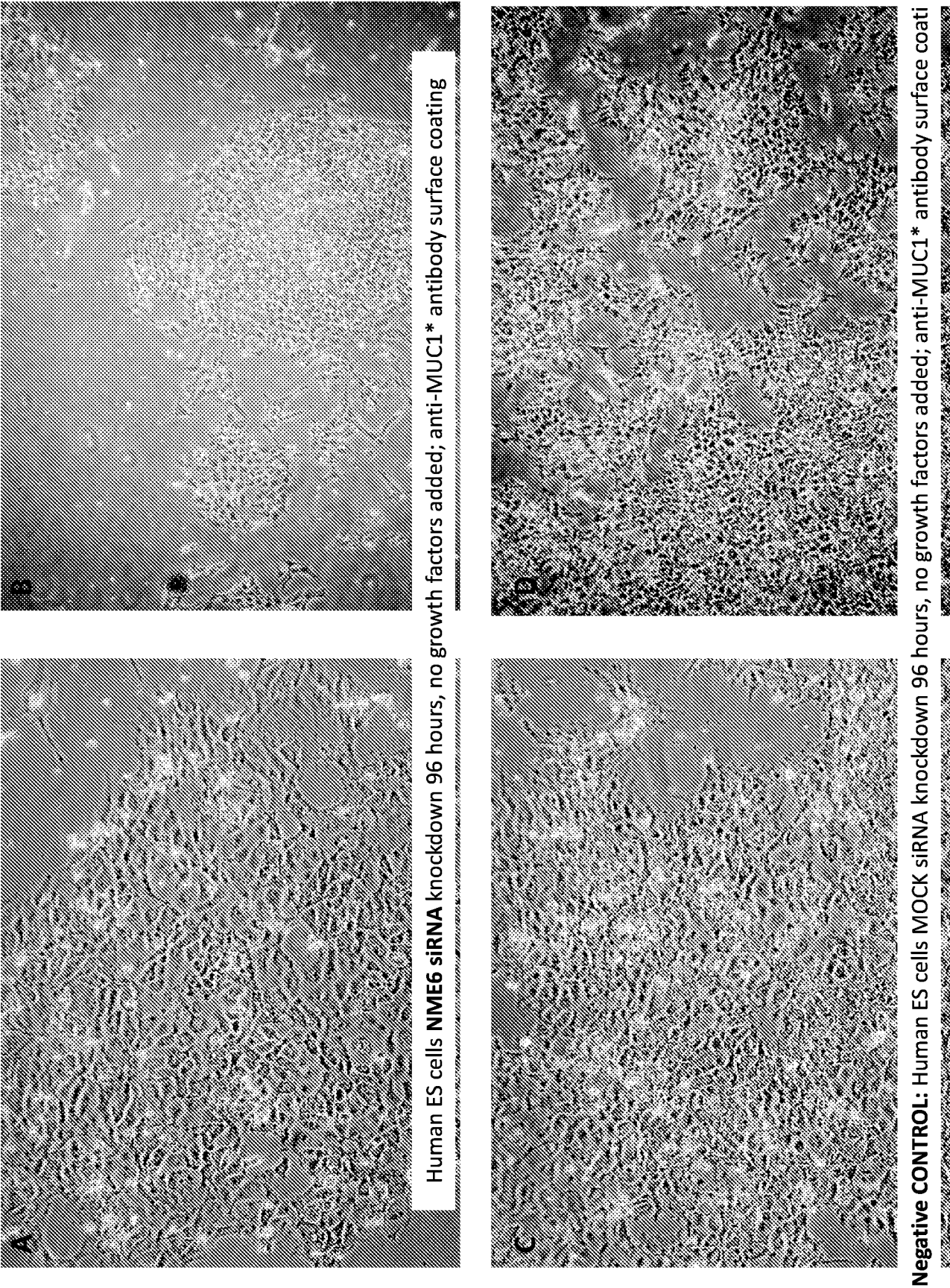


Figure 24

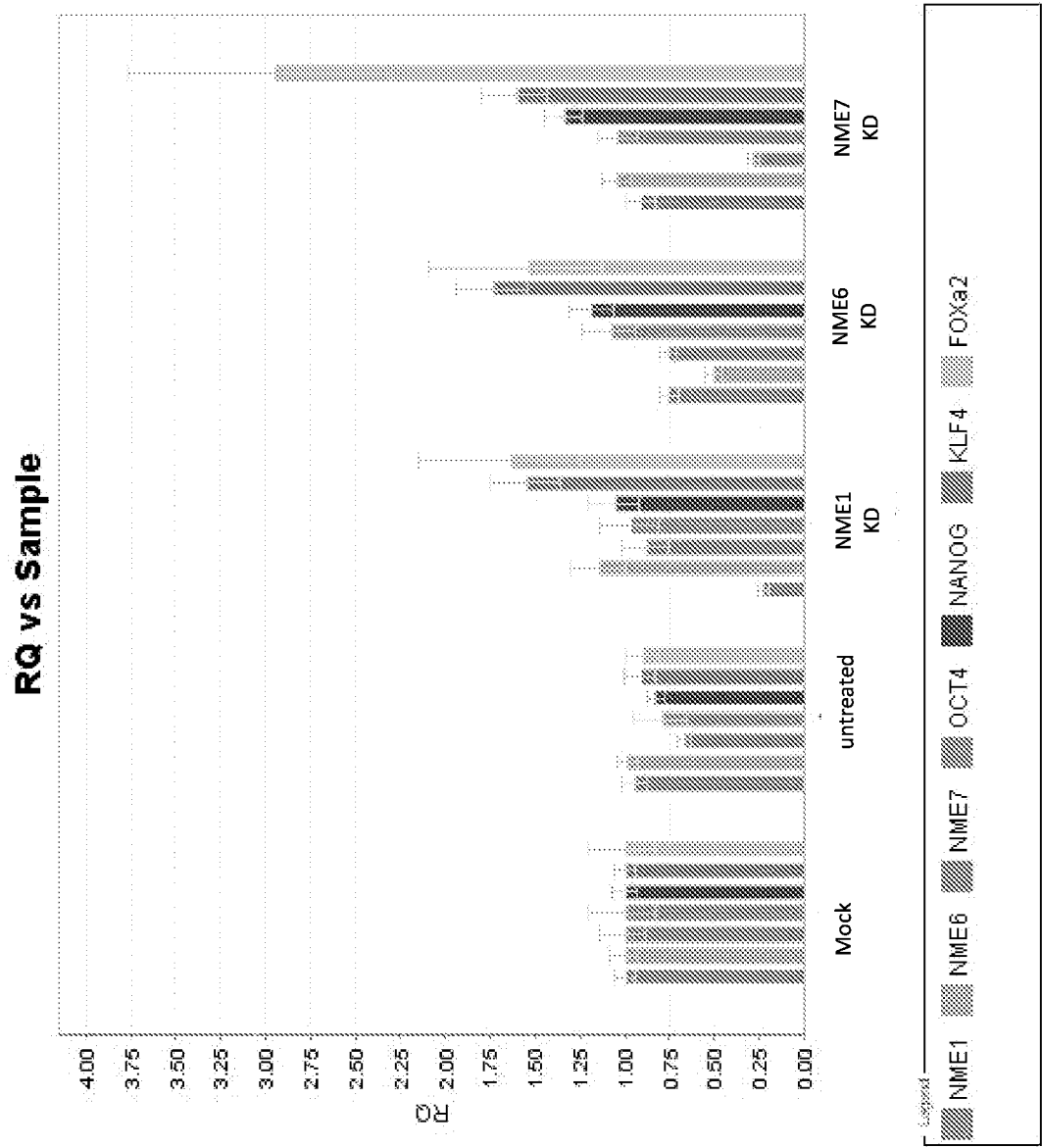


Figure 25

26/30

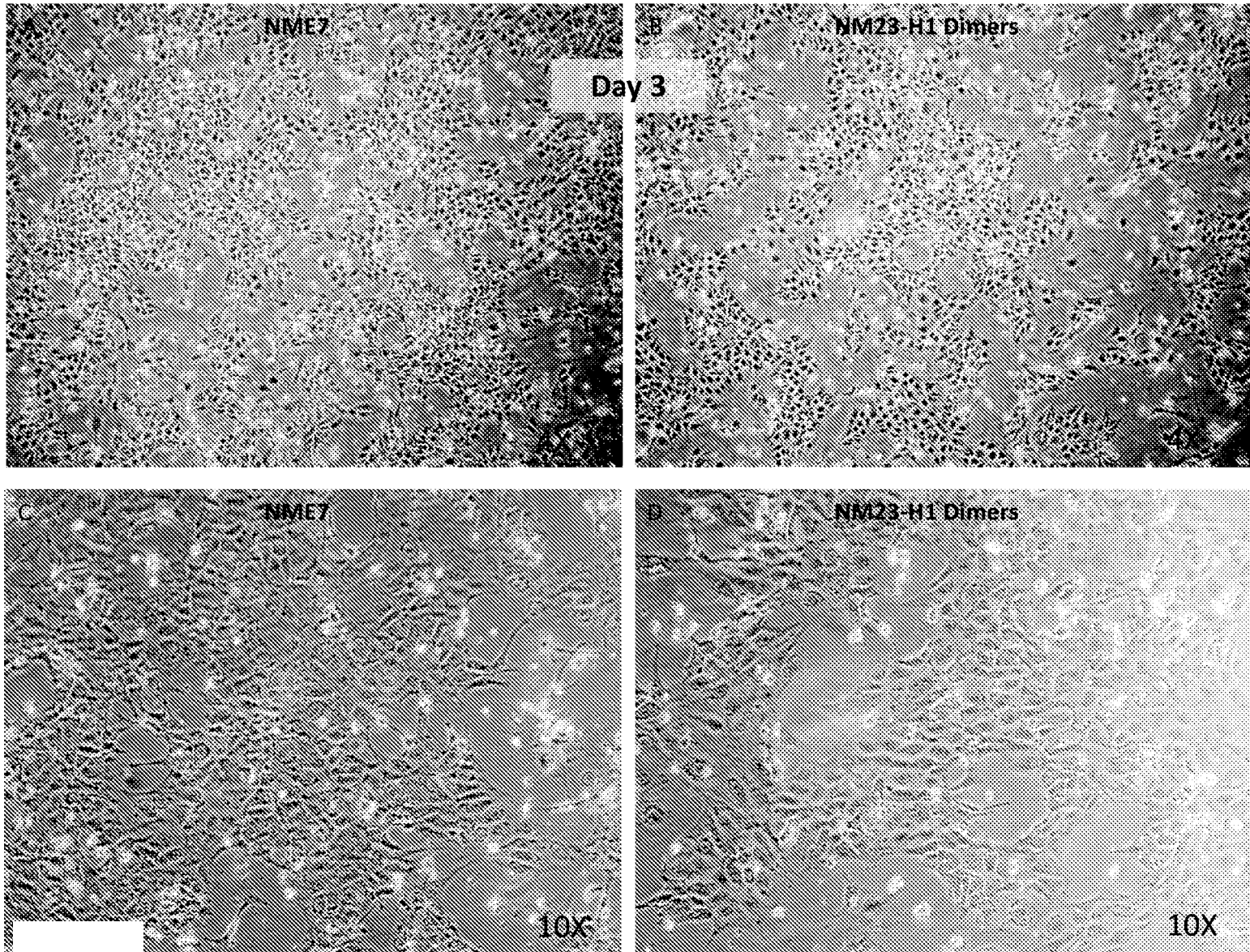


Figure 26

ELISA shows NME7 Dimerizes MUC1*

MUC1* extra cellular domain peptide immobilized on plate was bound by NME7 to saturation; a second MUC1* peptide with a C-terminal His-tag or Biotin tag was added and visualized by HRP labeled antibody to either His-tag or HRP labeled streptavidin

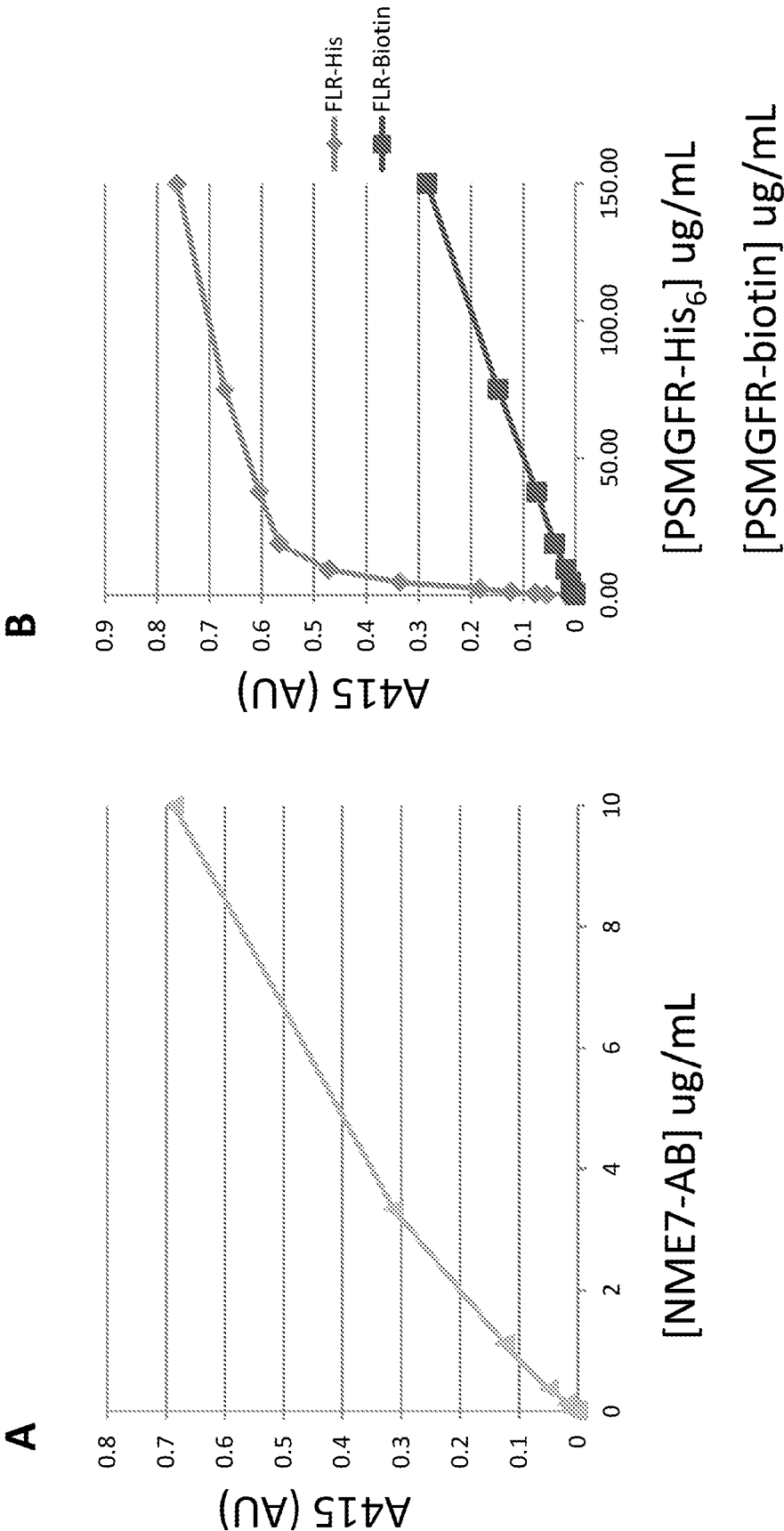


Figure 27

**T47D breast cancer cell growth assay – cells treated
with NME1 Hexamers**

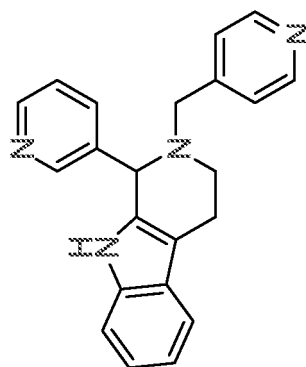
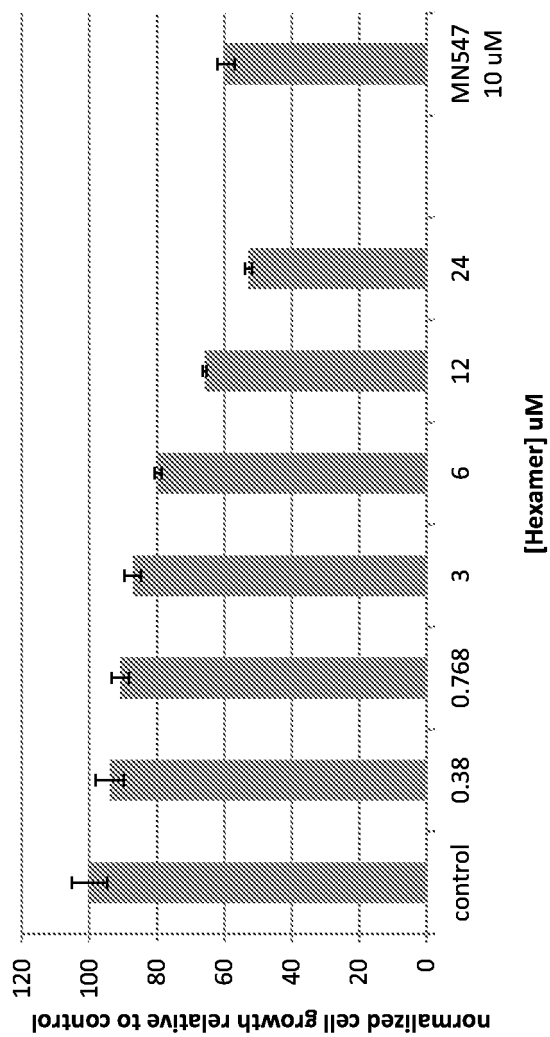
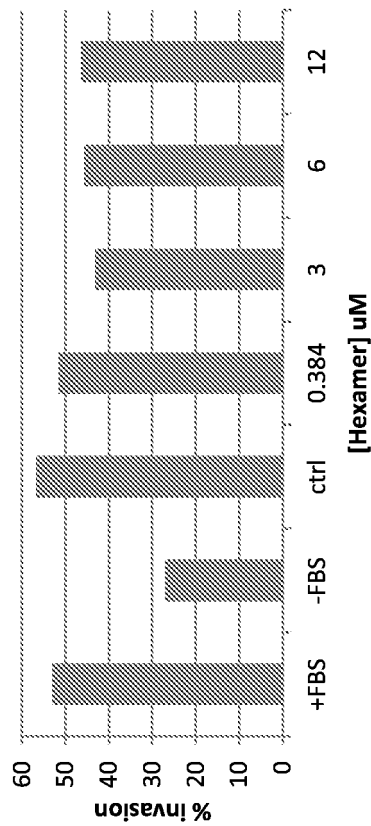


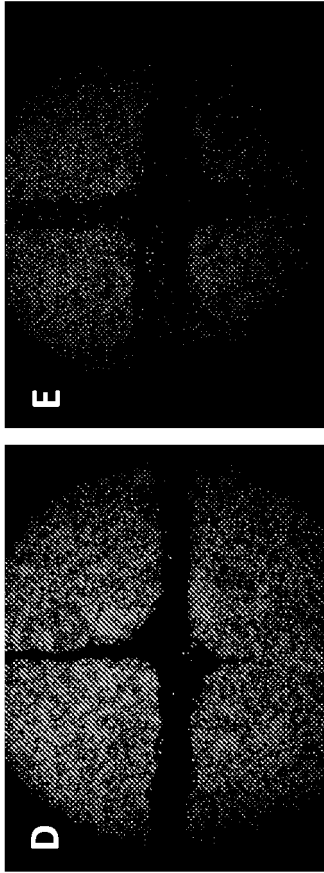
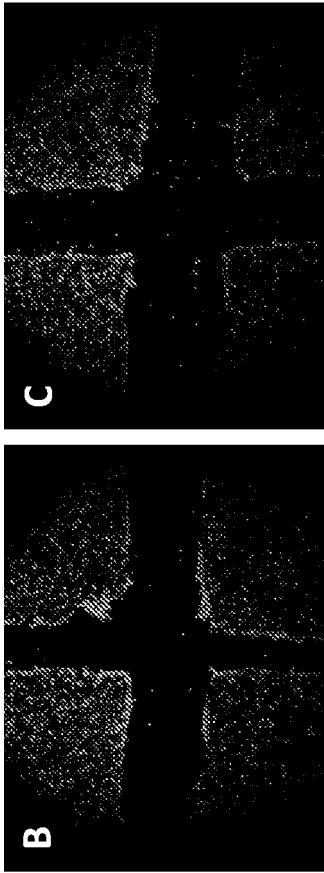
Figure 28

A

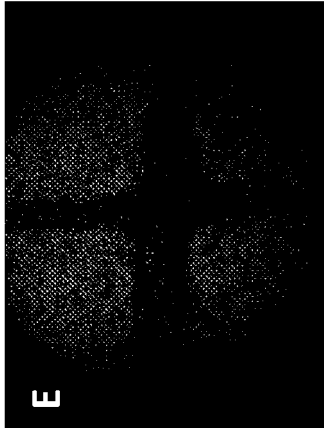
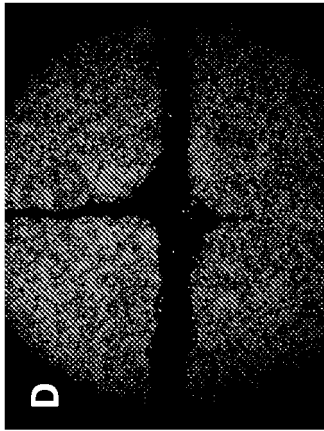
DU145 prostate cancer cell migration assay
– cells treated with NME1 Hexamers



T=0



T=16hr



ctrl

12 uM Hexamer

Figure 29

Figure 30

