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(54) Title: SHUTTLE AGENT PEPTIDES OF MINIMAL LENGTH AND VARIANTS THEREOF ADAPTED FOR TRANSDUCTION OF CAS9-RNP AND OTHER NUCLEOPROTEIN CARGOS

Fig. 4C

SEQ ID NO:	Peptide	Sequence	Cas9-RNP		Change*
			-	+	
266	FSD356	KWKLLKLSRLLKLWGSGGGSQAG	74%	51%	-23%
267	FSD357	KWKLLKLSRLLKLWGSGGGSRRR	59%	4%	-55%
370	FSD446	KWKLLKLSRLLKLWGSGGGSHTSDQCN	68%	34%	-34%
35	FSD250	KWKLLKLSRLLKLWGSGGGSARAAEQAE	73%	13%	-60%
207	FSD296	KWKLLKLSRLLKLWQGGSGGGSQAEAAEQAE	80%	4%	-76%
169	FSD246	KWKLLKLSRLLKLWGSGGGSYARAAEQAE	83%	1%	-82%
174	FSD251	KWKLLKLSRLLKLWGSGGGSAAEQAE	43%	36%	-7%

*Change in GFP transduction efficiency in the presence (+) or absence (-) of Cas9-RNP

(57) Abstract: Compositions and methods for delivering nucleoprotein cargos such as Cas9-RNP genome editing and ABE-Cas9-RNP base editing complexes to the cytosolic/nuclear compartment of eukaryotic cells via synthetic peptide shuttle agents are described herein. Also described herein are shortened synthetic peptide shuttle agents having a length of less than 20 amino acids having defined geometries associated with cargo transduction activity. The synthetic peptide shuttle agents are peptides comprising an amphipathic alpha-helical motif having both a positively-charged hydrophilic outer face and a hydrophobic outer face, wherein the synthetic peptide shuttle agent is independent from or is not covalently linked to the cargos. Shuttle agents engineered for increased resistance to inhibition by nucleoproteins and/or extracellular DNA/RNA are also described herein.

[Continued on next page]

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