Supporting Information:

Protein delivery using Cys_2-His_2 zinc-finger domains

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Keywords: protein delivery / cell-penetrating peptide / zinc-finger protein /

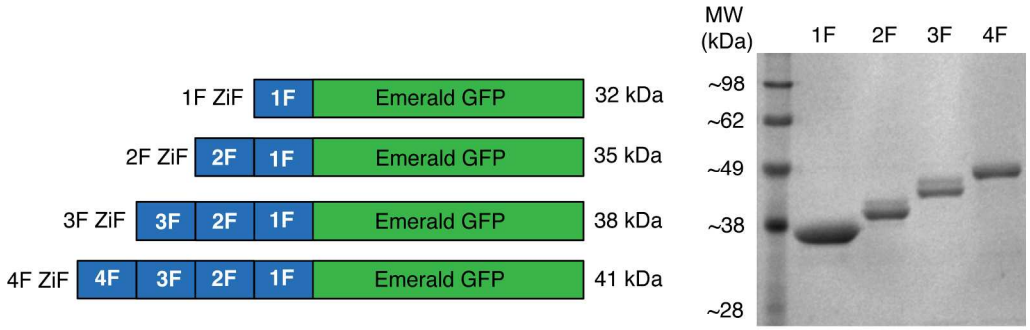

Figure S1. Doman organization **(left)** and SDS-PAGE **(right)** of purified one-, two-, three-, and four-finger ZiF-Emerald GFP protein.

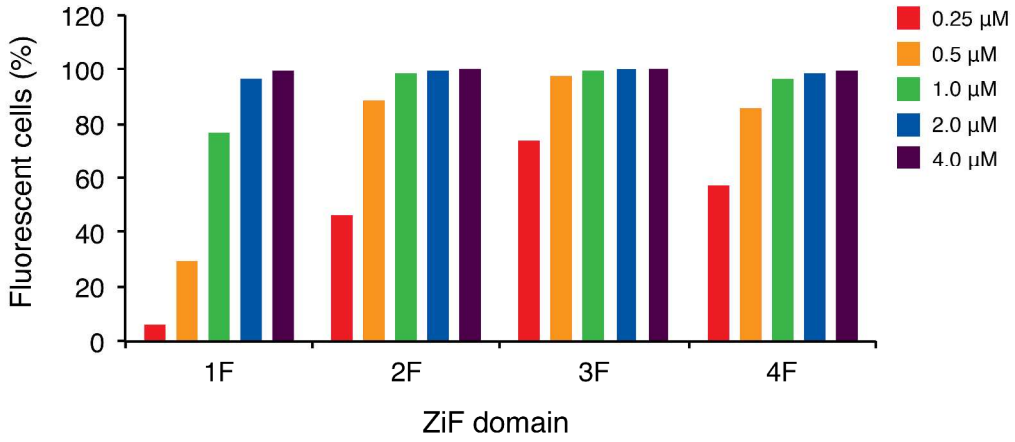

Figure S2. **ZiF-mediated protein uptake into mammalian cell types.** Percentage of fluorescent HeLa cells as determined by flow cytometry after treatment with increasing concentrations of one-, two-, three- and four-finger ZiF-EmGFP protein.

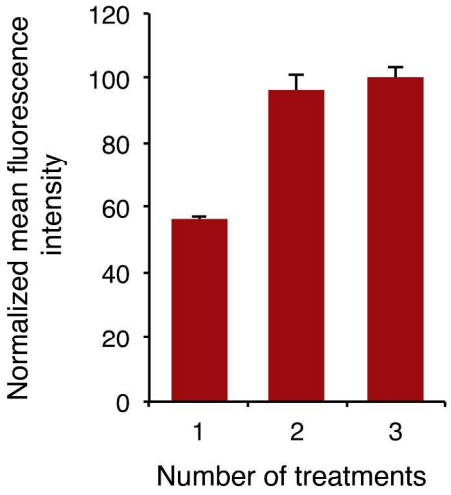

Figure S3. Consecutive treatments increase the efficiency of ZiF protein uptake. Normalized mean fluorescent intensity as determined by flow cytometry in HeLa cells treated with 2.0 μM of two-finger ZiF-EmGFP protein. Cells were incubated with protein for 90 min at 37 °C. Error bars indicate standard deviation (n = 3).

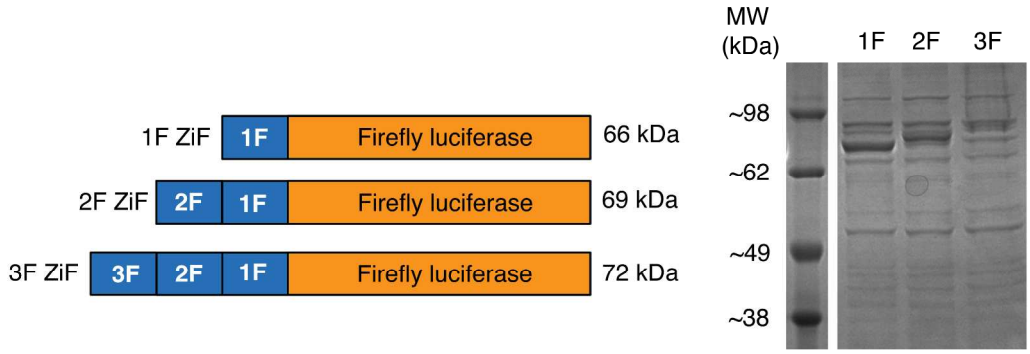

Figure S4. Doman organization **(left)** and SDS-PAGE **(right)** of purified one-, two-, and three-finger ZiF-luciferase protein.

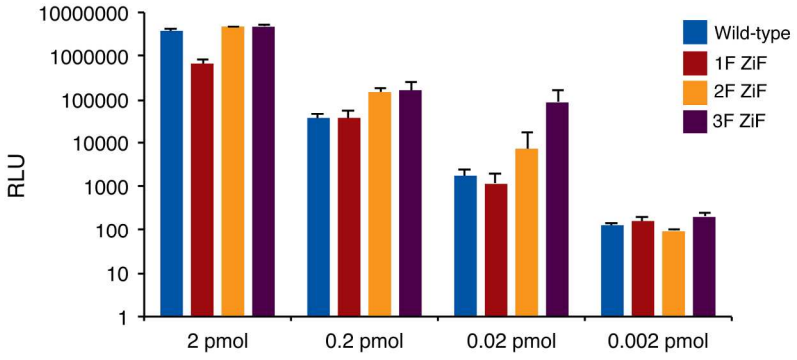

Figure S5. ZiF domain fusion does not reduce luciferase activity. Relative luminescence units (RLU) of purified one-, two- or three-finger ZiF-luciferase and wild-type (unmodified) luciferase protein. Error bars indicate standard deviation (n = 3).

>5' GFP NdeI
GGAAATTGCATATGAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGC

>5' TAT-GFP NdeI
GGAAATTGCATATGGGTCGCAAAAAGCGTCGTCAACGCCGTCGCGGAGGTAGCGGAGGTAGCAAGGGCGA
GGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGC

>5' HSV-GFP NdeI
GGAAATTGCATATGGATGCCGCAACAGCGACCCGTGGACGTAGCGCCGCCTCTCGTCCGACTGAACGTCC
ACGTGCCCCTGCCCGCTCCGCGTCTCGCCCGCGGCGCCCGGTTGATAAGGGCGAGGAGCTGTTCACCGGG
GTGGTGCCCATCCTGGTCGAGC

>3' GFP Sac1
GGGCTTTGTTAGCAGCCGGATCTCAGTGGT

>5' AgeI-ZF-Luc
GCTCACACCGGTATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGCGCCAT

>5' NdeI-wt-Luc
TTTCTTTTTCGGCATATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGCGCCAT

>3' Sac1-Luc
CGACGGATCTGAGCTCTTATTGTCGACTTTACACGGCGATCTTTCCGCCCTTCTTGGCCTTTATGAGGAT
CTCTCTGATTTTTCTTGCGTCGAGTTTT

>5' Ub-Fwd
GGATCTTCATATGACCGGTATGCAGATTTTTGTGAAAACCCTGACCGGCAAAACCATTACCCTGGAAGTG
GAACCGAGCGATACCATTGAAAACGTGAAAGCGAAAATTCAGGATAAAGAAGGCATTCCGCCGGATCAGC
AGCGCCT

>3' Ub-Rev
AGGCATTCCGCCGGATCAGCAGCGCCTGATTTTTGCGGGCAAACAGCTGGAAGATGGCCGCACCCTGAGC
GATTATAACATTCAGAAAGAAAGCACCCTGCATCTGGTGCTGCGCCTGCGCGGCGGCACCGGT CATATG
GGATCTT

Table S1. Primers used in this study.

>1F-ZiF-Emerald GFP

MGHHHHHHMPKKKRKVLEPGEKPYKCPECGKSFSASAALVAHQRTHTGVSKGEELFTGVVPILVELDGDV
NGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGY
VQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIK
VNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLG
MDELYKTS

>2F-ZiF-Emerald GFP

MGHHHHHHMPKKKRKVLEPGEKPYKCPECGKSFSASAALVAHQRTHTGEKPYKCPECGKSFSASAALVAH
QRTHTGVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTL
TYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDG
NILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQ
SKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKTS

>3F-ZiF-Emerald GFP

MGHHHHHHMPKKKRKVLEPGEKPYKCPECGKSFSASAALVAHQRTHTGEKPYKCPECGKSFSASAALVAH
QRTHTGEKPYKCPECGKSFSASAALVAHQRTHTGVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGD
ATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY
KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSV
QLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKTS

>4F-ZiF-Emerald GFP

MGHHHHHHMPKKKRKVLEPGEKPYKCPECGKSFSASAALVAHQRTHTGEKPYKCPECGKSFSASAALVAH
QRTHTGEKPYKCPECGKSFSASAALVAHQRTHTGEKPYKCPECGKSFSASAALVAHQRTHTGVSKGEELF
TGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHM
KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH
KVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHM
VLLEFVTAAGITLGMDELYKTS

>TAT-Emerald GFP

MGHHHHHHMGRKKRRQRRRGGSGGSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFI
CTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGD
TLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTP
IGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKTS

>HSV-Emerald GFP

MGHHHHHHMDAATATRGRSAASRPTERPRAPARSASRPRRPVDKGEELFTGVVPILVELGGDVNGHKFSV
SGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIF
FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRH
NIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKT
S

>1F-ZiF-Luciferase

MGHHHHHHMPKKKRKVLEPGEKPYKCPECGKSFSASAALVAHQRTHTGMEDAKNIKKGPAPFYPLEDGTA
GEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPV
LGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQ
SMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQII
PDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFSFFAKSTLID
KYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAK
VVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLI

KYKGYQVAPAELESILLQHPNIFDAGVAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKL
RGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKIAV

>2F-ZiF-Luciferase

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EAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVFVSKKGLQ
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PKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFL
RSLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTE
TTSAILITPEGDDKPGAVGKVVPFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDK
DGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGVAGLPDDDAGELPAAV
VVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKIAV

>3F-ZiF-Luciferase

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>1F-ZiF-Ub-Emerald GFP

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TAAGITLGMDELYKTS

>2F-ZiF-Ub-Emerald GFP

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>3F-ZiF-Ub-Emerald GFP

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PPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGTGVSKGEELFTGVVPILVELDGDVNGHKFS
VSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTI
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TS

>4F-ZiF-Ub-Emerald GFP

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FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK
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>5F-ZiF-Ub-Emerald GFP

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KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHY
QQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKTS

>6F-ZiF-Ub-Emerald GFP

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CGKSFSASAALVAHQRTHTGEKPYKCPECGKSFSASAALVAHQRTHTGMQIFVKTLTGKTITLEVEPSDT
IENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGTGVSKGEELFTGVVPI
LVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFF
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DKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKTS

Table S2. Amino acid sequences of proteins used in this study. ZiF domains are highlighted orange. Ubiquitin domain is highlighted green.