

Supplemental Methods

Plasmid construction

The plasmids used in this study are outlined in Table S1 (*Additional file 10*). The primers used for cloning are displayed in Table S2 (*Additional file 11*).

Unless stated otherwise, proteins were expressed under control of a *tetA* promoter from vector pASK-IBA3 (IBA GmbH). In order to express fusion proteins comprising a signal sequence and hEGF, a chimera encoding ssHbp/hEGF was initially created *via* a three-round overlap-extension PCR procedure. In the first round, the gene encoding recombinant hEGF was amplified using pUC19-hEGF(3SS), which carries a synthetic hEGF-encoding sequence (Genbank accession number AF274587 with the following silent mutations: A15G; T30C and T75A) (gift from W. Quax, University of Groningen, The Netherlands), as a template. The forward primer used was ASAA_hEGF_fw and the reverse primer was *HindIII*_hEGF_rev. In the second round of PCR, the ssHbp coding sequence was amplified using pEH3-Hbp [1] as a template. The forward primer used was IBA_*XbaI*_Hbp_fw and the reverse primer used was ASAA_Hbp_rev. The ssHbp and hEGF coding sequences were fused in a third round of PCR. In this reaction the products from PCR rounds 1 and 2 were mixed and combined with the primers IBA_*XbaI*_Hbp_fw and reverse primer *HindIII*_hEGF_rev. The resulting product was cloned into pASK-IBA3 using the *XbaI* and *HindIII* restriction sites, generating pIBA-ssHbp/hEGF.

To create the remaining signal sequence/hEGF fusion constructs, cassettes encoding the signal sequences of TorA, PhoE, and DsbA were amplified by PCR. To generate the ssTorA-encoding cassette pC4Meth-100TorA/P2 [2] was used as a template in combination with primers IBA_*XbaI*_TorA_fw and ASAA_TorA_rev. The ssPhoE-encoding cassette was generated using pC4Meth-94PhoE [3] as the template and the primers IBA_*XbaI*_PhoE_fw and ASAA_Phoe_rev. To generate the ssDsbA cassette, *E. coli* TOP10F' genomic DNA was used as the template with the primers IBA_*XbaI*_DsbA_fw and ASAA_DsbA_rev. The resulting products were cloned into pIBA-Hbp/hEGF using the *XbaI* and *NheI* restriction sites, giving rise to pIBA-ssTorA/hEGF, pIBA-ssPhoE/hEGF and pIBA-ssDsbA/hEGF, respectively.

In order to create pIBA-signal sequence/PLA2 constructs, a fragment encoding the mature region of human PLA2(TypeV) was amplified by PCR. The template used was pET24as/PLA2V (gift from P. Cronet, Astrazeneca) carrying a human PLA2(TypeV) encoding sequence (Genbank accession number NM_00029). The primers used were ASA_PLA2_fw and PLA2_HindIII_rev. The resulting product was cloned into pIBA-ssHbp/hEGF, pIBA-ssTorA/hEGF, pIBA-ssPhoE/hEGF and pIBA-ssDsbA/hEGF using the *NheI* and *HindIII* restriction sites, giving rise to pIBA-ssHbp/PLA2, pIBA-ssTorA/PLA2, pIBA-ssPhoE/PLA2 and pIBA-ssDsbA/PLA2, respectively.

In order to create pIBA-signal sequence/IL3 constructs, a fragment encoding the mature region of human IL3 was amplified by PCR using pLat-IL3 [4] as a template. The primers used were ASA_IL3 fw and IL3_HindIII_rev. The resulting product was cloned into pIBA-ssHbp/hEGF, pIBA-ssTorA/hEGF, pIBA-ssPhoE/hEGF and pIBA-ssDsbA/hEGF using the *NheI* and *HindIII* restriction sites, giving rise to pIBA-ssHbp/IL3, pIBA-ssTorA/IL3, pIBA-ssPhoE/IL3 and pIBA-ssDsbA/IL3, respectively.

Plasmids pIBA-TrxA, pIBA-ssTorA/TrxA, pIBA-MBP, pIBA-ssTorA/MBP, pIBA-SymE and pIBA-ssTorA/SymE were all constructed by PCR. To construct pIBA-TrxA plasmid pMO2 [5] as a template along with the primers *XbaI*-RBS-TrxA fw and *HindIII*-TrxA rev. The resulting PCR fragment was cloned into plasmid pIBA-ssTorA/Pla2 using the *XbaI* and *HindIII* restriction sites, generating pIBA-TrxA. For construction of pIBA-ssTorA/TrxA plasmid pMO2 was used as a template. The primers used were ASA_TrxA_fw and *HindIII*_TrxA_rev. The resulting PCR fragment was cloned into plasmid pIBA-ssTorA/Pla2 using the *NheI* and *HindIII* restriction sites, giving rise to pIBA-ssTorA/TrxA. To construct pIBA-MBP *E. coli* MG1655 genomic DNA was used as a template along with the primers *XbaI*_RBS_MBP_fw carrying an *XbaI* site and the *HindIII*_MBP_rev. The PCR fragment was cloned into plasmid pIBA-ssTorA/Pla2 using the *XbaI* and *HindIII* restriction sites, giving rise to pIBA-MBP. To create the plasmid pASK-IBA-TorA/MBP, *E. coli* MG1655 genomic DNA was used as a template and the primers used were ASA_MBP fw and *HindIII*_MBP rev. The resulting PCR fragment was cloned into plasmid pIBA-ssTorA/Pla2 using the *NheI* and *HindIII* restriction sites, resulting in pIBA-ssTorA/MBP. To create pIBA-SymE, *E. coli* MG1655 genomic DNA was used as the template along with the primers *XbaI*-RBS-SymE fw and *HindIII*_SymE rev. The resulting fragment was cloned into pIBA-

ssTorA/Pla2 using *XbaI/HindIII* sites, generating pIBA-SymE. To create pIBA-ssTorA/SymE, *E. coli* MG1655 genomic DNA was used as the template. The primers used were ASA_SymE_fw and HindIII_SymE_rev. The resulting PCR product was cloned into pIBA-ssTorA/PLA2 using the *NheI/HindIII* sites, giving rise to pIBA-ssTorA/SymE.

Plasmid pIBA-TrxA/ssTorA was created using a three-step overlap-extension PCR procedure. In the first step, the gene encoding TrxA was amplified using pIBA-ssTorA/TrxA as a template plus the primers *XbaI*_RBS_TrxA_fw and C_ssTorA_TrxA_rv. In the second step, the ssTorA coding sequence was amplified using pIBA-ssTorA/TrxA as a template along with the primers C_ssTorA_fw and *HindIII*_STOP_ssTorA_rv. In the third step, the TrxA and ssTorA encoding sequences were fused. In this reaction the products from PCRs 1 and 2 were mixed and combined with the primers *XbaI*_RBS_TrxA_fw and *HindIII*_STOP_ssTorA_rv. The resulting product was cloned into pIBA-TrxA using the *XbaI/HindIII* sites, resulting in pIBA-TrxA/ssTorA.

Plasmids encoding fusions carrying two or three ssTorA sequences at the N-terminus of the target protein were created by PCR as follows. The ssTorA encoding sequence was amplified with flanking *NheI* sites using pASK-IBA-ssTorA/Pla2 as a template. The primers used were ASA_*NheI*_ssTorA_rep_fw and ASA_*NheI*_ssTorA_rep_rev. The resulting fragment (*NheI*-ssTorA-*NheI*) was cloned into the single *NheI* site of pIBA-TorA/TrxA, generating pIBA-ssTorA(2x)/TrxA, carrying two ssTorA coding sequences in frame with the TrxA coding sequence. Cloning of the fragment *NheI*-ssTorA-*NheI* into the single *NheI* site of pIBA-ssTorA/MBP yielded pIBA-ssTorA(2x)/MBP and pIBA-ssTorA(3x)/MBP, carrying two or three ssTorA coding sequences in frame with the MBP coding sequence, respectively. To create a pIBA-ssTorA/TrxA derivative carrying three ssTorA-encoding sequences, an ssTorA-encoding PCR fragment was generated carrying flanking *SpeI* and *NheI* sites (*SpeI*-ssTorA-*NheI*). Plasmid pIBA-ssTorA/TrxA was used as a template along with the primers ASA_*SpeI*_ssTorA_rep_fw and ASA_*NheI*_ssTorA_rep_rev. The resulting product was digested with *SpeI* and *NheI* and cloned into the single *NheI* site of pIBA-ssTorA/TrxA, yielding pIBA-ssTorA(3x;SN)/TrxA. To create plasmids carrying two or three ssTorA sequences at the C-terminus of TrxA, the digested *SpeI*-ssTorA-*NheI* fragment was cloned into the *NheI* site of pIBA-TrxA/ssTorA, yielding pIBA-TrxA/ssTorA(2x) and pIBA-TrxA/ssTorA(3x), carrying two or three ssTorA coding sequences in frame with the TrxA coding sequence, respectively.

In order to create plasmid pIBA-ssTorA(3x)/GFP, first plasmid pIBA-ssTorA(3x)/SUMO was created by PCR using pET-SUMO (Invitrogen) as a template. The primers used were *XbaI*_ssTorA_hisSUMO fw and *HindIII*-STOP-SUMO rv. The resulting product was digested with *XbaI* and *HindIII* and cloned into *NheI*/*HindIII*-digested pIBA-ssTorA(3x;SN)/TrxA, yielding pIBA-ssTorA(3x)/SUMO. Subsequently, the GFPmut2-encoding region was amplified by PCR using pTHV038 [6] as a template in combination with the primers *NheI*-GFP fw and *HindIII*-GFP rv. The resulting product was cloned into pIBA-ssTorA(3x)/SUMO using the *NheI*/*HindIII* restriction sites, yielding pIBA-ssTorA(3x)/GFP.

Plasmid pBAD24-TorA(HA) was constructed by PCR using *E. coli* MG1655 genomic DNA as the template and the primers TorA_*EcoRI*_fw and TorA-HA_*Sall*-rev. Part of the PCR product (*torA**) was digested with *EcoRI* and the resulting 2306 bp fragment was cloned into the single *EcoRI* site of pBAD24 [7], yielding pBAD24-TorA(HA)-E/E. Subsequently, part of the PCR product *torA** was digested with *SfuI* and *Sall* generating a 2354 bp fragment that was cloned into the *SfuI*/*Sall* sites of pBAD24-TorA(HA)-E/E, yielding pBAD24-TorA(HA).

Plasmid pEH3-ssTorA/Hbp was created using a three-round PCR procedure. First a PCR was carried out using pBAD24-TorA(HA) as a template and the primers pEH_*XbaI*-TorA fw and ssTorA(Hbp) rv. Second, a PCR was carried out using pEH3-Hbp [1] as a template and the primers Hbp(ssTorA) fw and Hbp(mat) rv. Third, a fusion PCR was carried out using the products from step 1 and 2 as templates together with the primers pEH_*XbaI*-TorA fw and Hbp(mat) rv. The resulting product was cloned into the *XbaI*/*XmaI* sites of pEH3-Hbp, yielding pEH3-ssTorA/Hbp. All DNA sequences were confirmed using semi-automated DNA sequencing.

References

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