Supporting Information – S1 File

Table A. Primers used for cloning *Ec*GReg and *Bpe*GReg in pTrc99A.

Primer name	Primer sequence (5'-3')
*Ec*GReg SacI top	AAGAGCTCATGGAGATGTATTTTAAAAG
*Ec*GReg BamHI bot	AAGGATCCCTAAAGACTGGCTTTCCAG
*Bpe*GReg SacI top	AAGAGCTCTTGAAGCCTTCGCCTGAAATC
*Bpe*GReg BamHI bot	AAGGATCCCTAGTGCTGGCCGGACTG

Table B. Primers used for the construction of *Ec*GReg mutants and truncated *Ec*GReg.

Primer Name	Primer Sequence 5'-3'
Primers for globin domain mutants	
*Ec*GReg F42A mutF	CATTATCTGAGTATCGAGGCCTATCGAATTGTCCGC
*Ec*GReg F42A mutR	GCGGACAATTCGATAGGCCTCGATACTCAGATAATG
*Ec*GReg Y43A mutF	CTGAGTATCGAGTTTGCCCGAATTGTCCGCATCG
*Ec*GReg Y43A mutR	CGATGCGGACAATTCGGGCAAACTCGATACTCAG
*Ec*GReg A68T mutF	GCGGCAGTTGAAGAGTACCATGGAACGCTGGATTATTAAC
*Ec*GReg A68T mutR	GTTAATAATCCAGCGTTCCATGGTACTCTTCAACTGCCGC
*Ec*GReg M69A mutF	CAGTTGAAGAGTGCGGCCGAACGCTGGATTATTAAC
*Ec*GReg M69A mutR	GTTAATAATCCAGCGTTCGGCCGCACTCTTCAACTG
Primers for DGC domain mutants	
*Ec*GReg G374A mutF	GATTATGTTTTCCGCTACGCCGGCGATGAATTTATCATTG
*Ec*GReg G374A mutR	CAATGATAAATTCATCGCCGGCGTAGCGGAAAACATAATC
*Ec*GReg G375A mutF	GTTTTCCGCTACGGGGCCGATGAATTTATCATTG
*Ec*GReg G375A mutR	CAATGATAAATTCATCGGCCCCGTAGCGGAAAAC
*Ec*GReg D376A mutF	GTTTTCCGCTACGGGGGCGCCGAATTTATCATTGTTTTG
*Ec*GReg D376A mutR	CAAAACAATGATAAATTCGGCGCCCCCGTAGCGGAAAAC
*Ec*GReg E377A mutF	CCGCTACGGGGGCGATGCCTTTATCATTGTTTTGAC
*Ec*GReg E377A mutR	GTCAAAACAATGATAAAGGCATCGCCCCCGTAGCGG
*Ec*GReg F378A mutF	CTACGGGGGCGATGAAGCCATCATTGTTTTGACTG
*Ec*GReg F378A mutR	CAGTCAAAACAATGATGGCTTCATCGCCCCCGTAG
*Ec*GReg L300A mutF	GTCGGTATGGATGTAGCCACGAAATTACTTAACC
*Ec*GReg L300A mutR	GGTTAAGTAATTTCGTGGCTACATCCATACCGAC
*Ec*GReg L300D mutF	GTCGGTATGGATGTAGACACGAAATTACTTAACC
*Ec*GReg L300D mutR	GGTTAAGTAATTTCGTGTCTACATCCATACCGAC
*Ec*GReg R306A mutF	GACGAAATTACTTAACGCCCGTTTCCTACCGACTATC
*Ec*GReg R306A mutR	GATAGTCGGTAGGAAACGGGCGTTAAGTAATTTCGTC
*Ec*GReg D333A mutF	GTCAGTGCTGATTATTGCCGTTGATAAATTCAAAG
*Ec*GReg D333A mutR	CTTTGAATTTATCAACGGCAATAATCAGCACTGAC
*Ec*GReg F337A mutF	GATTATTGACGTTGATAAAGCCAAAGAGATCAACGATAC
*Ec*GReg F337A mutR	GTATCGTTGATCTCTTTGGCTTTATCAACGTCAATAATC
*Ec*GReg K338A mutF	GACGTTGATAAATTCGCCGAGATCAACGATACG
*Ec*GReg K338A mutR	CGTATCGTTGATCTCGGCGAATTTATCAACGTC
*Ec*GReg N341A mutF	GATAAATTCAAAGAGATCGCCGATACGTGGGGCCATAAC
*Ec*GReg N341A mutR	GTTATGGCCCCACGTATCGGCGATCTCTTTGAATTTATC
*Ec*GReg D342A mutF	GATAAATTCAAAGAGATCAACGCCACGTGGGGCCATAAC

*Ec*GReg D342A mutR	GTTATGGCCCCACGTGGCGTTGATCTCTTTGAATTTATC
*Ec*GReg D350A mutF	GGCCATAACACTGGTGCCGAAATTCTGCGTAAAG
*Ec*GReg D350A mutR	CTTTACGCAGAATTTCGGCACCAGTGTTATGGCC
*Ec*GReg L353A mutF	CACTGGTGATGAAATTGCCCGTAAAGTCTCTCAGG
*Ec*GReg L353A mutR	CCTGAGAGACTTTACGGGCAATTTCATCACCAGTG
*Ec*GReg L353D mutF	CACTGGTGATGAAATTGACCGTAAAGTCTCTCAGG
*Ec*GReg L353D mutR	CCTGAGAGACTTTACGGTCAATTTCATCACCAGTG
*Ec*GReg D368A muF	CAACGTCCGCAGTAGTGCCTATGTTTTCCGCTAC
*Ec*GReg D368A mutR	GTAGCGGAAAACATAGGCACTACTGCGGACGTTG
*Ec*GReg D368K mutF	CAACGTCCGCAGTAGTAAATATGTTTTCCGCTAC
*Ec*GReg D368K mutR	GTAGCGGAAAACATATTTACTACTGCGGACGTTG
*Ec*GReg R372A mutF	GTAGTGATTATGTTTTCGCCTACGGGGGCGATG
*Ec*GReg R372A mutR	CATCGCCCCCGTAGGCGAAAACATAATCACTAC
Primers for middle domain mutants	
*Ec*GReg H223A mutF	GGCCTGTGGTTTAACGCCAAAGGTCGACATTATTTTAG
*Ec*GReg H223A mutR	CTAAAATAATGTCGACCTTTGGCGTTAAACCACAGGCC
*Ec*GReg K224A mutF	CCTGTGGTTTAACCATGCCGGTCGACATTATTTTAG
*Ec*GReg K224A mutR	CTAAAATAATGTCGACCGGCATGGTTAAACCACAGG
Primers for truncated *Ec*GReg	
*Ec*GReg 25-end SacI top	CGGAGCTCGCTAAAGCCGCGGAAATTG
*Ec*GReg 50-end SacI top	CGGAGCTCCCGCATGCCGAAGAATTC
*Ec*GReg 75-end SacI top	CGGAGCTCAACGTGCTTTCTGCCCAG
*Ec*GReg 100-end SacI top	CGGAGCTCCGCATAGGAATTCCGGTAG
*Ec*GReg 125-end SacI top	CGGAGCTCTCTTCGGATTATTCCGCC
*Ec*GReg 154-end SacI top	AAGAGCTCATGGCGTTTACCTTTAGTGAC
*Ec*GReg 268-end SacI top	AAGAGCTCATGTTTTTATTACAGATAAG

Table C. Primers used for the construction of *Bpe*GReg mutants and truncated *Bpe*GReg.

Primer name	Primer Sequence 5'-3'
Primers for *Bpe*GReg globin domain mutants	
*Bpe*GReg F42A mutF	GGCGCTGGCCGATTATGCCTACGAGTGCATGCTGG
*Bpe*GReg F42A mutR	CCAGCATGCACTCGTAGGCATAATCGGCCAGCGCC
*Bpe*GReg Y43A mutF	GCTGGCCGATTATTTCGCCGAGTGCATGCTGGCCG
*Bpe*GReg Y43A mutR	CGGCCAGCATGCACTCGGCGAAATAATCGGCCAGC
*Bpe*GReg S68A mutF	GACCAAGCTGCATGCCGCCATGCAGGATTGGCTGG
*Bpe*GReg S68A mutR	CCAGCCAATCCTGCATGGCGGCATGCAGCTTGGTC
*Bpe*GReg M69A mutF	CAAGCTGCATGCCTCCGCCCAGGATTGGCTGGAATC
*Bpe*GReg M69A mutR	GATTCCAGCCAATCCTGGGCGGAGGCATGCAGCTTG
Primers for *Bpe*GReg middle domain mutants	
*Bpe*GReg K226A mutF	CCTGTGGTTCATCCACGCCGCGGCGCACGCCTTCG
*Bpe*GReg K226A mutR	CGAAGGCGTGCGCCGCGGCGTGGATGAACCACAGG
Primers for truncated *Bpe*GReg	
*Bpe*GReg 156-475 SacI top	AAGAGCTCGCCTACTCGGTGTCGCAC
*Bpe*GReg 267-475 SacI top	AAGAGCTCATCCTGCACAGCGTGCGC
*Bpe*GReg 297-475 SacI top	AAGAGCTCGATACGCTGACCCGGCTG
*Bpe*GReg 1-155 tga BamHI bot	AAGGATCCTCAATGGCACATCATCTCGAC
*Bpe*GReg 1-266 tga BamHI bot	AAGGATCCTCAGGCCAGGCGCTGGTCGGG
*Bpe*GReg 1-296 tga BamHI bot	AAGGATCCTCAGCGCCCCGACTCCAGG

Table D. Primers used for expression of truncated *Bpe*GReg in pET-3a.

Primer name	Primer sequence (5'-3')
*Bpe*GReg Xa top	ATCGAGGGAAGGTTGAAGCCTTCGCCTGAAATC
Histidine Xa top	CACCACCACCACCACCACATCGAGGGAAGG
NdeI histidine top	AACATATGCACCACCACCACCACCAC
*Bpe*GReg BamHI bot	AAGGATCCCTAGTGCTGGCCGGACTG
*Bpe*GReg 1-155 tga BamHI bot	AAGGATCCTCAATGGCACATCATCTCGAC
*Bpe*GReg 1-266 tga BamHI bot	AAGGATCCTCAGGCCAGGCGCTGGTCGGG
*Bpe*GReg 1-296 tga BamHI bot	AAGGATCCTCAGCGCCCCGACTCCAGG

Figure A. Absorption spectra of truncated *Bpe*GReg proteins. a) Wild-type *Bpe*GReg (solid red line) showed heme-bound absorption spectra. b) *Bpe*GReg$_{155}$, *Bpe*GReg$_{266}$, and *Bpe*GReg$_{296}$ showed heme-bound absorption spectra, similar to that of wild-type *Bpe*GReg.

a. b.