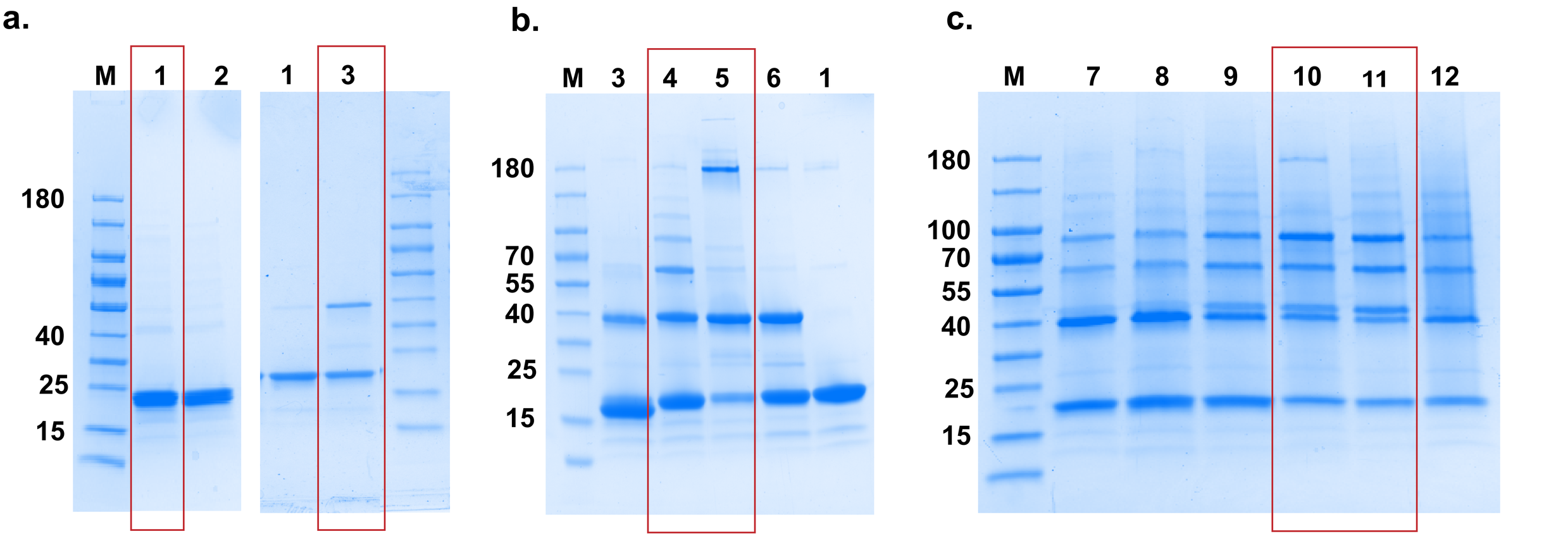
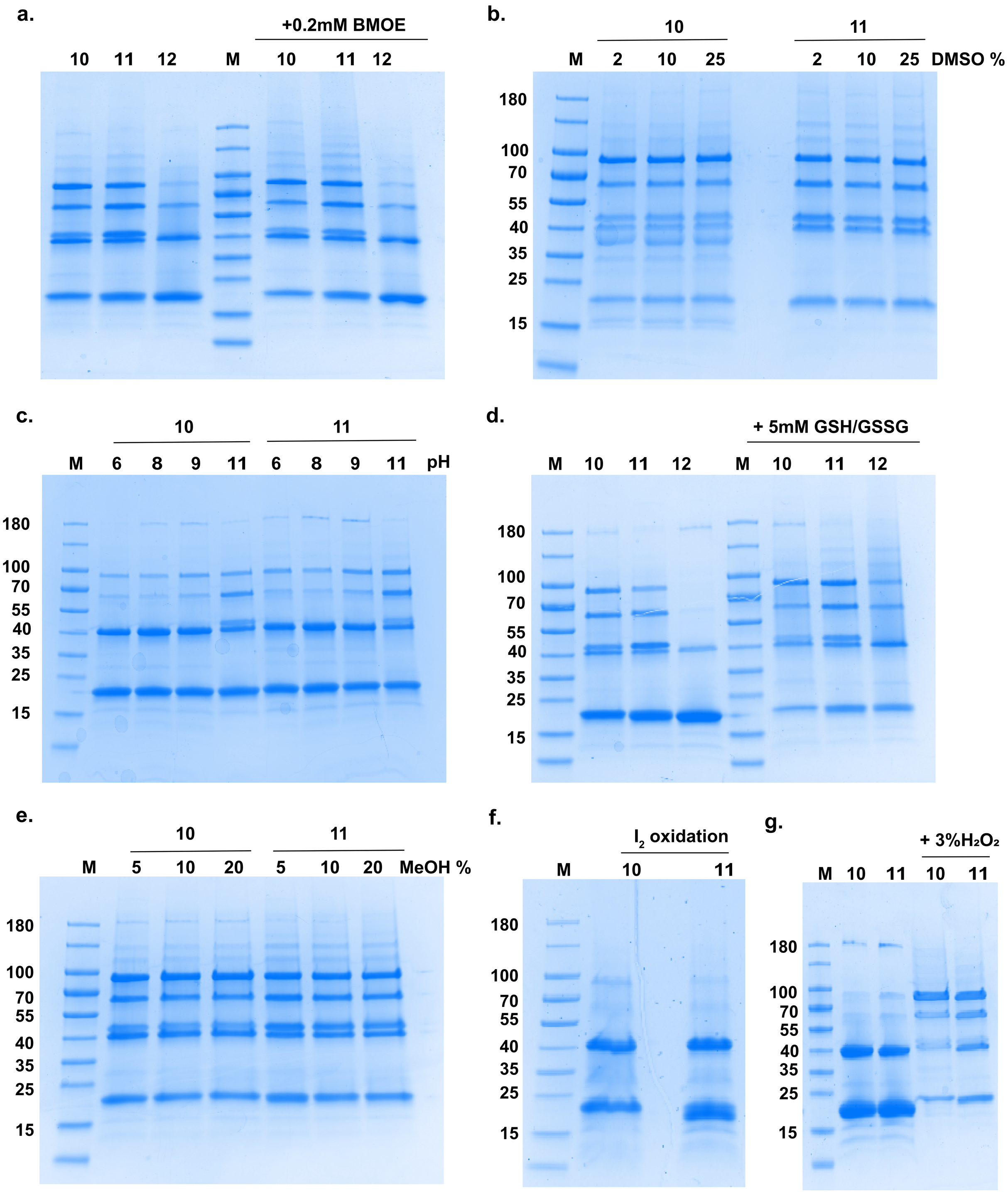


Figure S1. (a-d) Non-reducing SDS-PAGE for designed cysteine residue substitutions.



9. tES A152C - A74C - R66C
10. tES A152C - G67C
11. tES A152C - L53C - G67C
M. Marker

Figure S2. (a-g) Non-reducing SDS-PAGE to increase disulfide formation in 9,10, and 11 constructs.

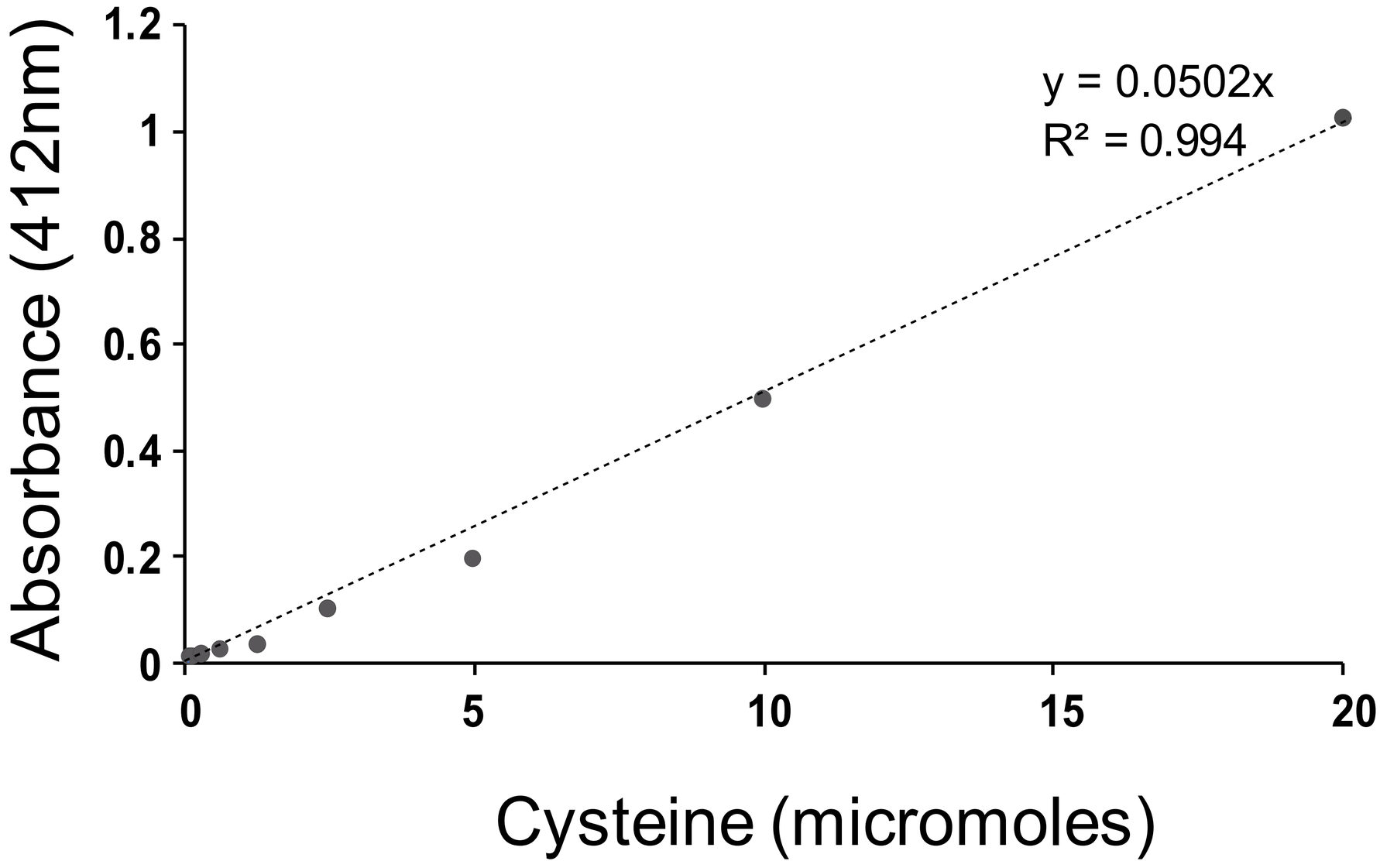


Figure S3. Ellman's Assay Standard Curve.

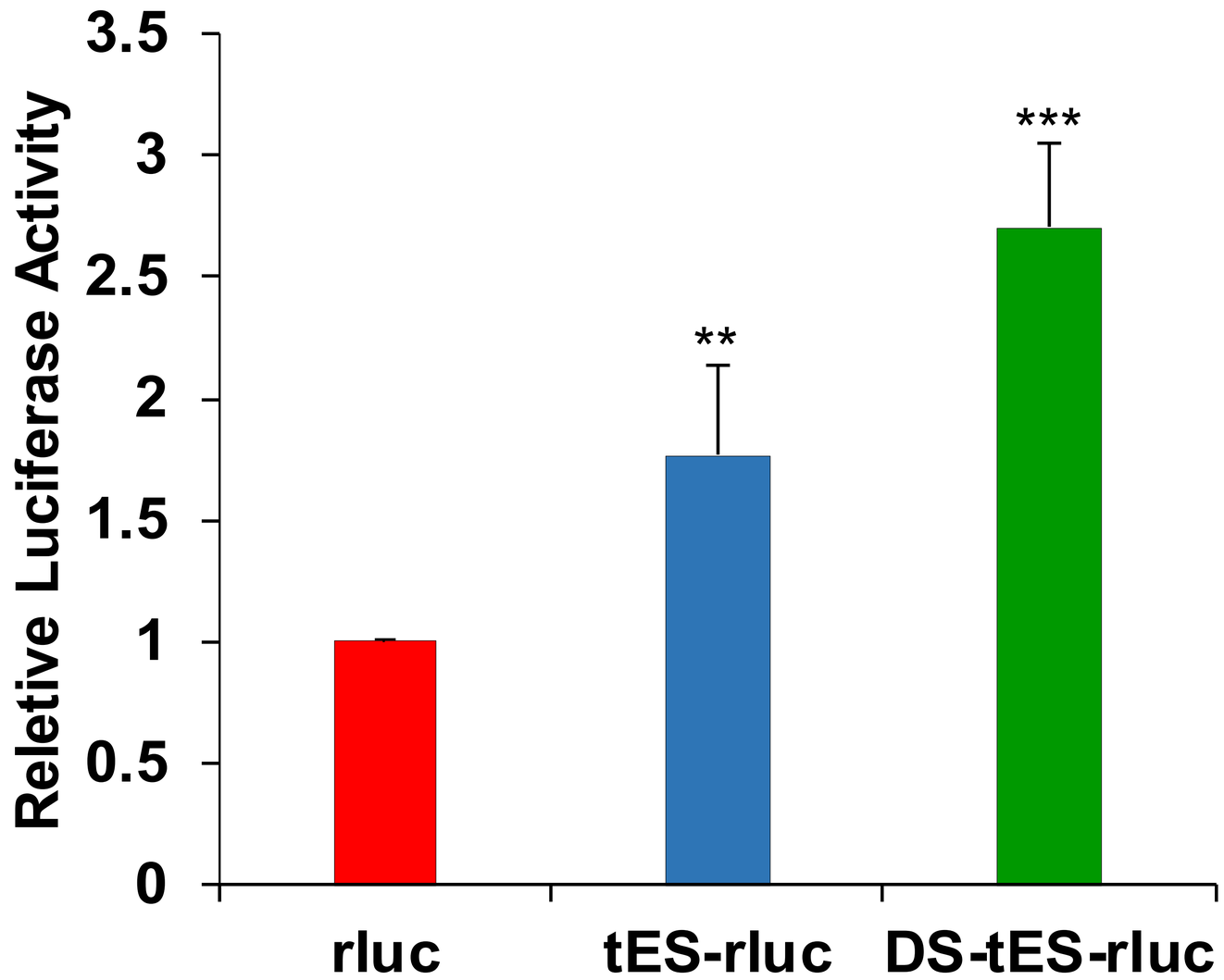
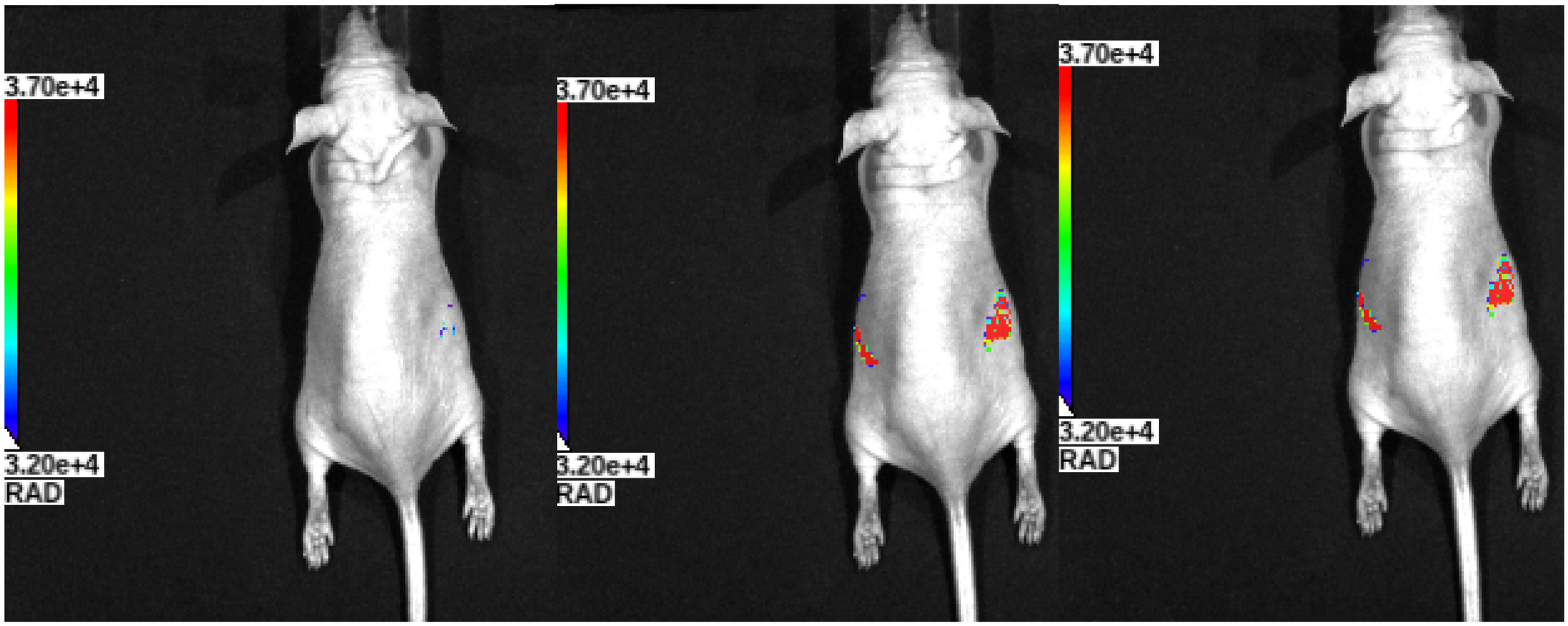


Figure S4. Permeability assay in Caco2 monolayers shows that both tES and DS-tES can permeabilize through intesti-nal epithelium. (Data are shown as mean ± SEM, n = 3. *** P < 0.001. and ** P< 0.01)

a.



b.

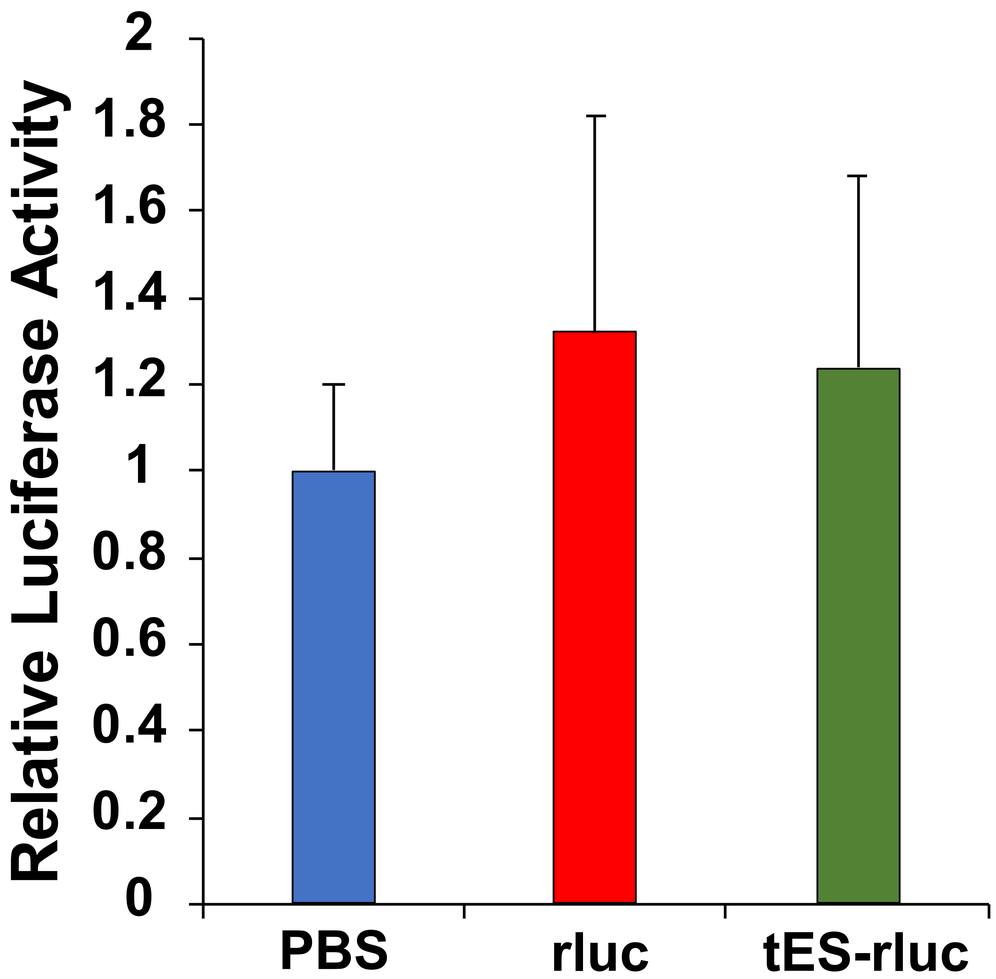


Figure S5.(a,b) Bioluminescence emitted from mice administered with tES-rluc/ rluc in 3 h.

Features	DS-tES	tES
Number of cysteine per subunit	5	0
Non-reducing SDS-PAGE	Majorly oxidized Shell	Subunits
Molecular diameter	~13 nm	~13 nm
Stable at acidic pH (~4)	Yes	No
Against pepsin digestion at acidic pH	Highly stable	Not stable
Permeable through Caco2 monolayer	High	High

Table S1. Comparative analysis of DS-tES and tES