	Construct Name	Primers (5'→3')
1	FLAG-STAM-1 (1-195)	Forward: GGAGGTCTATATAAGCAGAGC
		Reverse: ATATTCTAGATTAGGAAAGGGTGGTTGACTGCTG
2	FLAG-STAM-1 (1-269)	Forward: GGAGGTCTATATAAGCAGAGC
		Reverse: ATATTCTAGATTAAGTGAGATCTGCAGTCACAAA
3	FLAG-STAM-1 (1-390)	Forward: GGAGGTCTATATAAGCAGAGC
		Reverse: ATATTCTAGATTACTGATTCTGTAACTTTGCATA
4	FLAG-STAM1 (391-540)	Forward: ATATAAGCTTCCATATTATATGCAG
		Reverse: GGGCCAGGAGAGGCACTG
5	FLAG-STAM-1 (144-540/ ΔVHS)	Forward: ATATAAGCTTGCTATTGGCTCTCAGGCT
		Reverse: GGGCCAGGAGAGGCACTG
6	FLAG-STAM-1 (337-540)	Forward: ATATAAGCTTCACCAGATGGGACCTCTC
		Reverse: GGGCCAGGAGAGGCACTG
7.	FLAG-STAM-1 (212-540)	Forward: ATATAAGCTTGGCCGAAAAGTTCGTGC
		Reverse: GGGCCAGGAGAGGCACTG
8.	FLAG-STAM-1 (270-540)	Forward: ATATAAGCTTGCTGAACCAGAAATGATT
		Reverse: GGGCCAGGAGAGGCACTG
9.	FLAG-STAM-1- Delta GAT (Δ343-	Forward 1: TGTCACCAGATGGGACCTCTCGATCCGATGTATTCCATGTATGC
	377)	Reverse 1: GAGAGGTCCCATCTGGTGACA
		Forward 2: GGAGGTCTATATAAGCAGAGC
		Reverse 2: GGGCCAGGAGAGGCACTG
10.	GST-STAM-1-Delta GAT (Δ343-377)	Forward: ATATGAATTCTGCCTCTTTTTGCCACCAATCCC
		Reverse: ATATCTCGAGCTATAGCAGAGCCTTCTG
11.	GST-STAM-1-GAT (296-380)	Forward: ATATGAATTC TGGAGCCGGAACCAGCC
		Reverse: ATATCTCGAGCTACATCGGATCTTCGTTCATTAAC
12.	FLAG-STAM-1-GAT (296-380)	Forward: ATAT AAG CTT GAG CCG GAA CCA GCC
		Reverse: ATATTCTAGACTACATCGGATCTTCGTTCATTAAC
13.	YFP-STAM-1	Forward: ATATAAGCTTTGCCTCTTTTTGCCACCAATCCCTTC
		Reverse: ATATGGTACCCTACATCGGATCTTCGTTCATTAAC
14.	FLAG-Arr-2-(25-161)	Forward: ATATAAGCTTCGGGACTTTGTGGACCAC
		Reverse: CAAACAACAGATGGCTGGCAAC
15.	GST-Arr-2-(25-161)	Forward: ATATCCCGGGCGGGACTTTGTGGACCAC
		Reverse: ATATCTCGAGCTACCGCTTGTGGATCTTCTCCTCCA

Malik and Marchese Supplementary Table S1

Supplemental Figures

Supplemental Figure S1. (A-E) Equimolar amounts (~134 nM) of GST-arrestin-2 and GST immobilized on glutathione-Sepharose resin were incubated with lysates from HEK293 cells transiently transfected with various FLAG-STAM-1 constructs. Bound proteins were detected by immunoblotting using the anti-FLAG M2 antibody, followed by staining with Ponceau-S (B-E) or immunoblotting for GST (A) to assess the amount of GST fusion proteins used in the binding assays. Shown are representative blots from one of three independent experiments.

Supplemental Figure S2. Equimolar amounts (117 nM) of GST-STAM-1 and GST immobilized on glutathione-Sepharose resin were incubated with lysates from HEK293 cells transiently transfected with HA-arrestin-2 constructs. Bound proteins were detected by immunoblotting using the anti-HA antibody followed by staining with Ponceau-S to assess the amount of GST fusion proteins used in the binding assay. Shown are representative blots from one of three independent experiments.

Supplemental Figure S3: (A) EGFR degradation was assessed in HeLa cells transfected with FLAG-STAM-1-GAT, FLAG-Arr-2-(25-161) or pCMV. Cells were treated with 100 ng/ml EFG for 1 hr followed by immunobloting as described in Materials and methods. Shown are representative immunoblots from one of three independent experiments. (B) Bar graph represents that amount of EGFR degraded as compared to vehicle treated cells \pm S.E.M. from three independent experiments. Data were analyzed by one-way analysis of variance and were found not to be significantly different.

Malik and Marchese Supplementary Figure S1



Ponceau-S

Malik and Marchese Supplementary Figure S2



Ponceau-S

Malik and Marchese Supplementary Figure S3

