Inventory of Supporting Information

Figure S1, Figure S2, Figure S3, Figure S4, Figure S5, Table S1, Table S2

Figure S1. HasS domain organisation. TM represents transmembrane helix.



Figure S2. Purification of $HasS_{CTD}$. SDS PAGE of inclusion bodies (1) solubilized in 8 M Urea, elution profile and SDS PAGE of fractions (2) collected from the size exclusion column.







Figure S4. Variation of the fluorescence emission of $HasS_{CTD}$ upon titration with aliquots of a solution of HasR signaling domain (square) at 1 mM and ribunclease A (triangle) at 1.1 mM. The excitation and emission wavelengths were 297 and 350 nm, respectively.



Figure S5. Residues of the signaling domain of HasR involved in the interaction with $HasS_{CTD}$ are in cyan and those affected by microsecond to millisecond time scale motions (Rex higher than 2.5 s⁻¹) are in red.



Table S1: HasS $_{\rm CTD}$ chemical shift (in ppm)

| | | Н | N | C | CA | СВ |
|-----|-----|------|--------|--------|-------|-------|
| | Met | 8.36 | 118.81 | 177.69 | 55.99 | 32.99 |
| 240 | Gly | 8.20 | 109.17 | 176.62 | 45.56 | - |
| 241 | Asp | 8.11 | 120.47 | 174.14 | 55.26 | 41.35 |
| 242 | Asp | 8.23 | 119.84 | 176.58 | 55.22 | 41.28 |
| 243 | Ala | 8.06 | 122.64 | 177.03 | 54.64 | 18.97 |
| 244 | Trp | 7.91 | 116.66 | 178.76 | 58.50 | 29.02 |
| 245 | Arg | 7.63 | 118.49 | 176.66 | 57.83 | 30.65 |
| 246 | Arg | 7.82 | 117.71 | 178.97 | 57.05 | 30.68 |
| 247 | Gly | 8.03 | 107.47 | 177.14 | 46.32 | - |
| 248 | Trp | 7.91 | 119.42 | 174.09 | 58.48 | 29.54 |
| 249 | Leu | 7.58 | 117.83 | 175.77 | 55.66 | 41.97 |
| 250 | Val | 7.48 | 117.03 | 176.68 | 62.85 | 32.40 |
| 251 | Ile | 7.64 | 119.90 | 175.65 | 61.14 | 39.05 |
| 252 | Asp | 8.01 | 121.98 | 174.83 | 54.54 | 41.83 |
| 253 | Arg | 7.98 | 117.88 | 175.70 | 57.21 | 30.72 |
| 254 | Gln | 8.29 | 119.86 | 176.68 | 56.70 | 27.90 |
| 257 | Thr | 8.00 | 112.80 | 178.03 | 66.26 | 68.48 |
| 258 | Gln | 7.93 | 120.50 | 176.95 | 58.56 | 28.64 |
| 259 | Ala | 7.91 | 121.87 | 178.15 | 55.22 | 18.37 |
| 260 | Leu | 8.02 | 116.20 | 179.27 | 57.53 | 41.55 |
| 261 | Ala | 7.76 | 120.54 | 178.55 | 54.77 | 18.22 |
| 262 | Gln | 7.70 | 115.91 | 180.17 | 57.78 | 28.51 |
| 263 | Leu | 7.85 | 118.69 | 177.91 | 57.27 | 41.87 |
| 264 | Asn | 7.84 | 115.32 | 177.76 | 54.99 | 38.98 |
| 265 | Arg | 7.55 | 117.76 | 176.23 | 57.01 | 30.40 |
| 266 | Tyr | 7.65 | 119.20 | 176.50 | 58.16 | 38.54 |
| 267 | Arg | 7.93 | 121.28 | 175.70 | 57.08 | 30.60 |
| 268 | Gly | 7.82 | 107.32 | 176.44 | 45.70 | - |
| 269 | Thr | 7.69 | 112.95 | 174.02 | 62.36 | 69.71 |
| 270 | Arg | 8.07 | 122.62 | 174.11 | 56.42 | 30.72 |
| 271 | Ile | 8.01 | 121.18 | 176.02 | 61.82 | 38.62 |
| 272 | Val | 7.73 | 119.80 | 175.45 | 61.80 | 32.83 |
| 273 | Ala | 7.98 | 125.70 | 174.90 | 52.33 | 19.64 |
| 274 | Val | 7.76 | 117.28 | 176.87 | 61.93 | 33.06 |
| 275 | Asn | 8.15 | 121.85 | 175.06 | 51.62 | 39.11 |
| 277 | Ala | 7.98 | 120.93 | 176.66 | 53.00 | 18.92 |
| 278 | Leu | 7.68 | 118.54 | 174.54 | 55.13 | 42.38 |
| 279 | Arg | 7.86 | 119.86 | 176.83 | 56.37 | 30.85 |
| 280 | Thr | 7.91 | 114.14 | 176.47 | 62.52 | 69.62 |
| 281 | Arg | 8.20 | 122.12 | 174.72 | 56.89 | 30.80 |
| 282 | Thr | 7.90 | 114.15 | 176.47 | 62.52 | 69.86 |
| 283 | Val | 7.95 | 119.81 | 176.83 | 62.98 | 32.38 |
| 284 | Ser | 8.08 | 116.79 | 175.90 | 59.43 | 63.78 |
| 285 | Gly | 8.14 | 109.55 | 175.14 | 45.91 | - |
| 286 | Val | 7.72 | 118.52 | 174.58 | 63.77 | 32.39 |
| 287 | Phe | 7.76 | 118.27 | 175.36 | 57.79 | 39.24 |
| 288 | Ala | 7.61 | 122.49 | 174.58 | 52.35 | 19.36 |
| 289 | Leu | 7.90 | 119.86 | 177.09 | 55.63 | 42.36 |
| 290 | Asn | 8.20 | 116.98 | 176.61 | 54.08 | 39.03 |

| 291 | Lys | 7.83 | 118.55 | 175.03 | 56.06 | 32.98 |
|-----|-----|------|--------|--------|-------|-------|
| 292 | Leu | 8.11 | 121.87 | 176.62 | 56.38 | 42.24 |
| 293 | Asp | 8.18 | 118.38 | 177.10 | 55.50 | 41.24 |
| 294 | Asp | 7.95 | 119.69 | 174.62 | 55.41 | 41.17 |
| 295 | Gly | 8.21 | 108.00 | 177.59 | 46.43 | - |
| 296 | Val | 7.96 | 118.68 | 174.54 | 64.59 | 32.00 |
| 297 | Gly | 8.29 | 108.87 | 177.12 | 46.59 | - |
| 298 | Ala | 7.85 | 122.99 | 175.07 | 53.89 | 18.98 |
| 299 | Ile | 7.78 | 117.61 | 178.97 | 63.08 | 38.15 |
| 300 | Arg | 8.16 | 121.03 | 176.66 | 58.53 | 30.39 |
| 301 | Gln | 8.02 | 118.86 | 177.54 | 57.70 | 29.10 |
| 302 | Glu | 8.01 | 119.86 | 177.13 | 57.65 | 29.84 |
| 303 | Leu | 7.95 | 119.14 | 177.32 | 55.87 | 42.06 |
| 304 | Ser | 7.77 | 113.69 | 177.04 | 59.04 | 63.97 |
| 305 | Ala | 8.02 | 124.22 | 174.89 | 53.48 | 19.20 |
| 306 | Arg | 7.89 | 117.41 | 178.00 | 56.82 | 30.57 |
| 307 | Gln | 7.86 | 116.92 | 176.11 | 56.23 | 29.24 |
| 308 | Leu | 7.91 | 120.43 | 175.29 | 54.69 | 42.75 |
| 309 | Asn | 8.02 | 119.15 | 177.54 | 52.82 | 39.17 |
| 310 | Leu | 7.91 | 121.80 | 173.97 | 52.86 | 42.60 |
| 312 | Gly | 8.18 | 107.05 | 176.23 | 45.88 | - |
| 313 | Ile | 7.69 | 118.54 | 177.66 | 61.77 | 39.01 |
| 315 | Leu | 7.82 | 121.88 | 174.36 | 55.59 | 42.63 |
| 317 | Tyr | 7.22 | 121.79 | 174.80 | 58.95 | 39.92 |
| | | | | | | |

Table S2. Summary of the NMR constraints used for the structure calculation, the restraint violations and structural statistics for the ensemble of 20 best conformers of the HasR signaling domain

| NOE-derived constraints: | 1141 | Energies (kcal/mol) | | |
|--|-----------------|--|--------------------|--|
| unambiguous: | 943 | E _{noe} | 15.5 ± 3.5 | |
| intraresidue | 420 | E _{bond} | 15.2 ± 0.9 | |
| sequential | 235 | E _{angle} | 90.4 ± 6.4 | |
| medium range | 80 | E_{vdv} | -349.5 ± 19.2 | |
| long range | 208 | E _{overall} | -3524.0 ± 52.3 | |
| ambiguous: | 198 | Mean pairwise RMSD (Å) residues 8-82: | | |
| intraresidue | 102 | Backbone atoms: | 1.41 ± 0.39 | |
| sequential | 45 | All heavy atoms: | 2.21 ± 0.40 | |
| medium range | 11 | For the residues with regular 2ndary structure | | |
| long range | 40 | Backbone atoms: | 0.81 ± 0.27 | |
| Hydrogen bonds: | 24 | All heavy atoms: | 1.52 ± 0.25 | |
| $DANGLE (\varphi, \psi)$: | 176 | | | |
| Total number of experimental. restraints | 1341 | Ensemble Ramachandran plot residues 8-82: | | |
| | | most favoured | 83.7% | |
| Residual distance constraint violations: | | additional allowed | 16.7% | |
| >=0.5 Å | 0.28 ± 0.70 | generously allowed | 0.0% | |
| >=0.3 Å | 0.87 ± 1.14 | disallowed | 0.0% | |