Table S4. Composition of peptidoglycan isolated from cells with defects in scaffolding proteins. Strains MT257 (Δ*bacA*), MAB238 (*mreB::mreB*^{sw}), and NA1000 (WT) were grown for 24 h in M2G^{-P} prior to analysis. Strains CJW2747 (Δ*rodZ::Ω* Pxyl::Pxyl-*rodZ*) and LS3809 (Δ*mreB* Pxyl::Pxyl-*mreB*) were first cultivated for 7 h in PYE and then grown for 24 h in M2G^{-P}. Values are the mean ± variance of two independent experiments.

Muropeptide	Relative percentage (%) in strain				
	WT	Δ*bacA*	RodZ depl.	MreB depl.	MreB^{sw}
Tri	0.9±0.0	1.4±0.0	5.0±0.0	5.1±0.1	3.8±0.0
Tetra	17.5±0.0	19.6±0.0	17.7±0.2	17.4±0.0	19.2±0.1
Penta	7.9±0.1	8.0±0.1	8.3±0.0	8.7±0.0	7.3±0.0
TriTri (LD)	0.2±0.0	0.2±0.0	0.8±0.0	1.0±0.0	0.5±0.0
TetraTri (LD)	1.1±0.0	0.7±0.0	1.9±0.0	2.5±0.0	1.9±0.0
TriAnh/TetraTri (LD)	0.9±0.0	1.1±0.0	3.6±0.0	3.7±0.0	3.0±0.0
TetraTetra (LD)	0.2±0.0	0.4±0.0	1.1±0.0	1.3±0.0	0.9±0.0
TetraTetra	15.1±0.0	16.8±0.2	13.6±0.0	13.3±0.1	15.8±0.0
TetraPenta	7.3±0.2	7.7±0.0	6.4±0.0	6.8±0.0	5.9±0.0
TetraTetraTri or TetraTetraTri (LD)	0.4±0.0	0.3±0.0	0.6±0.0	0.7±0.0	0.6±0.0
TetraAnh	0.3±0.0	0.5±0.0	0.9±0.0	0.8±0.0	0.8±0.0
TetraTetraTetra	4.4±0.1	5.0±0.0	3.7±0.0	3.1±0.0	4.4±0.0
TetraTetraPenta	1.8±0.0	1.9±0.0	1.4±0.0	1.3±0.0	1.3±0.0
TetraTetraTetraTetra	0.8±0.0	0.9±0.0	0.3±0.0	0.3±0.0	0.2±0.0
TetraTetraTetraPenta	0.4±0.0	0.4±0.0	0.1±0.0	0.2±0.0	0.1±0.0
PentaAnh	0.8±0.0	0.9±0.0	0.6±0.0	0.5±0.0	0.7±0.0
TetraTetraAnh	4.3±0.0	4.2±0.0	0.7±0.0	0.7±0.0	0.6±0.0
TetraPentaAnh	0.9±0.0	0.8±0.0	2.2±0.0	2.2±0.0	2.2±0.0
TetraTetraTriAnh or TetraTetraTri(LD)Anh	n.d.	n.d.	1.5±0.0	1.5±0.0	1.4±0.0
TetraTetraTetraAnh	7.6±0.1	7.0±0.0	5.5±0.0	4.9±0.1	6.1±0.0
TetraTetraPentaAnh	6.4±0.5	6.1±0.0	4.3±0.1	4.1±0.0	4.7±0.0
TetraTetraTetraPentaAnh I	1.2±0.0	1.3±0.0	0.6±0.0	0.4±0.0	0.7±0.0
TetraTetraTetraPentaAnh II	1.3±0.0	1.3±0.0	0.6±0.0	0.5±0.0	0.7±0.0
TetraTetraTetradiAnh	1.1±0.0	1.0±0.0	1.0±0.0	0.7±0.0	0.9±0.0
TetraTetraTetraPentadiAnh	0.9±0.0	0.9±0.0	1.3±0.0	1.0±0.0	1.3±0.0
TetraTetradiAnh	1.5±0.0	1.2±0.0	1.3±0.0	1.1±0.0	1.2±0.0
TetraTetraPentadiAnh	0.7±0.0	0.6±0.0	0.4±0.0	0.4±0.0	0.3±0.0
all known	85.7±1.7	90.2±0.0	85.3±0.1	84.4±0.2	86.4±0.1
Monomers (total)	32.9±0.5	35.1±0.0	38.2±0.6	38.6±0.3	36.8±0.2
Dimers (total)	35.7±1.7	35.4±0.0	36.9±0.1	38.7±0.0	36.8±0.0
Trimers (total)	26.0±1.5	24.3±0.0	21.5±0.2	19.7±0.1	22.8±0.1
Tetramers (total)	5.4±0.6	5.2±0.0	3.4±0.0	2.9±0.0	3.5±0.0
Tripeptides (total)	3.1±0.0	3.5±0.0	10.8±0.0	11.8±0.4	8.5±0.1
Tetrapeptides (total)	77.1±0.2	77.0±0.0	70.6±0.0	69.1±0.6	74.3±0.0
Pentapeptides (total)	19.5±0.1	18.9±0.0	18.6±0.0	19.1±0.0	17.2±0.0
3-3 crosslinks	1.0±0.0	0.8±0.0	3.0±0.0	3.7±0.0	2.6±0.0
Chain ends (Anh)	15.0±0.2	14.1±0.0	11.3±0.0	10.4±0.0	11.4±0.1
Degree of cross-linkage	39.2±0.6	37.8±0.0	35.3±0.2	34.7±0.1	36.3±0.1
% peptides in cross-links	67.1±0.5	64.9±0.0	61.8±0.6	61.4±0.3	63.2±0.2
	Mean of disaccharides units ± standard deviation (n=2)				
Average glycan chain length	6.7±0.0	7.1±0.0	8.8±0.0	9.6±0.0	8.8±0.0