Molecular Cell, *Volume 39*

Supplemental Information

An Smc3 Acetylation Cycle Is Essential for Establishment of Sister Chromatid Cohesion

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Figure S1

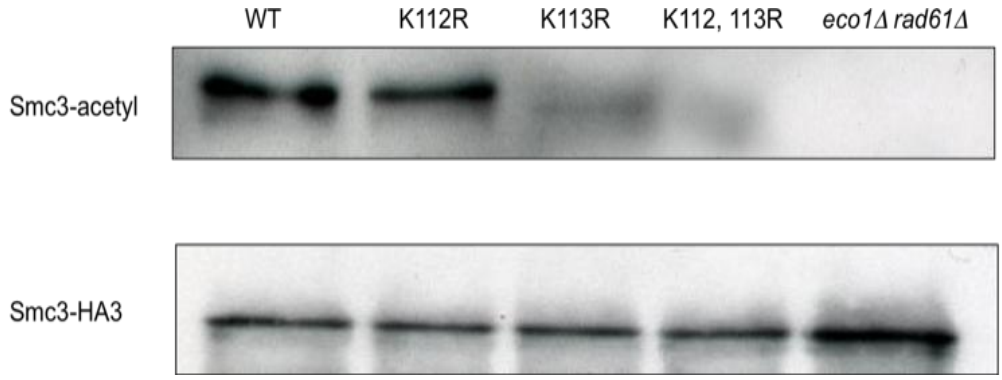

Figure S1. Specificity of a mouse antibody raised against the acetylated lysines K112 and K133 of Smc3. EU3444 (*SMC3: URA3: SMC3-3HA*), EU3445 (*SMC3: URA3: smc3 (K112R)-3HA*), EU3446 (*SMC3: URA3: smc3 (K113R)-3HA*), EU3447 (*SMC3: URA3: smc3 (K112R, K113R)-3HA*) and 15794 (*Δsmc3::HIS3, leu2::SMC3-HA3::LEU2 eco1Δ rad61Δ)* were grown in YPD. Cell extracts were prepared, and wild type and mutant Smc3-HA was immunopurified under denaturing conditions. The acetylation status of Smc3 was analyzed by Western blot using a mouse antibody raised against the acetylated lysines K112 and K133 of Smc3. Equal recovery of Smc3 in the immunoprecipitates was confirmed by probing the membrane with an antibody raised against the HA epitope tag fused to Smc3**.**

Figure S2

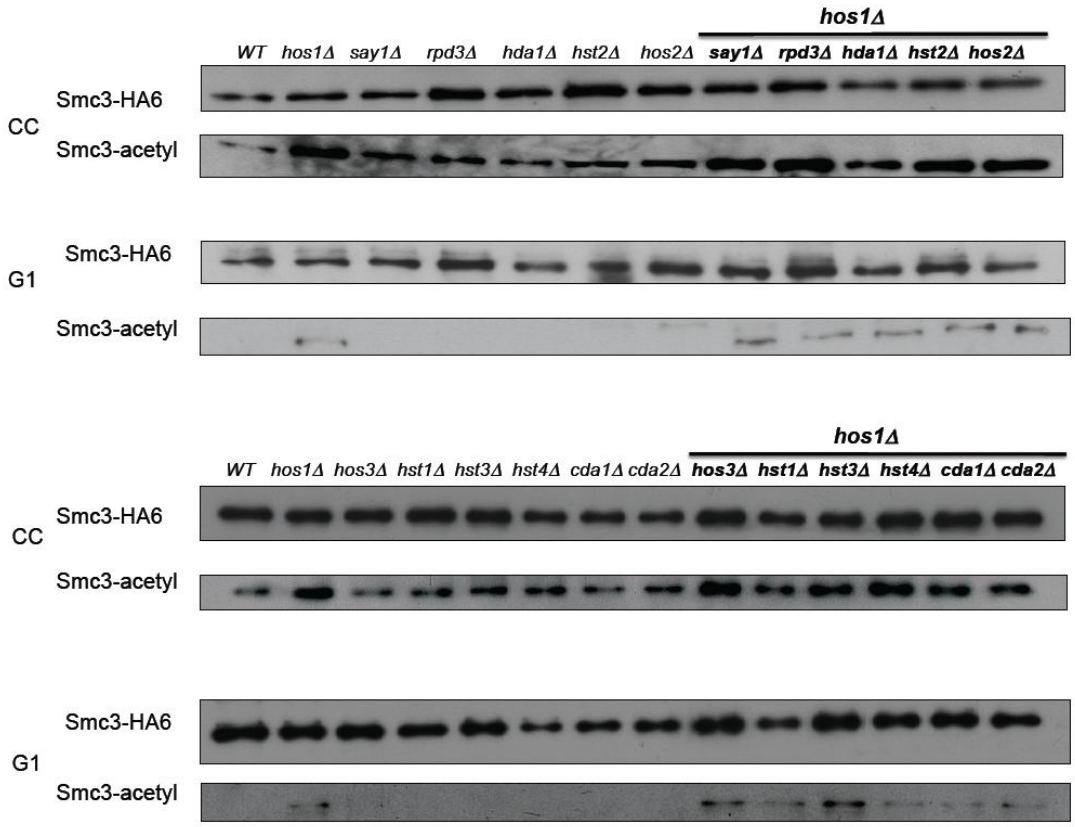

Figure S2. Hos1 is the main deactylase that deacetylates Smc3. In order to check if other deacetylases contribute to Smc3 deacetylation, eleven deacetylase genes have been individually inactivated in *hos1Δ* cells. Double and single mutants were grown in YPD and arrested in G1 by α factor. Proteins from these strains were extracted and the level of Smc3 acetylation monitored by Western blot of crude extracts using α-Smc3 acetylated antibodies.

Table S1. Strains Used

K7040	*MATa, ade2-101, CFIII (CEN3. L.YPH278) TRP1, SUP11*
K8965	*MATa esp1-1, pGAL1-10-ESP1::TRP1*
K8967	*MATa esp1-1, pGAL1-10-esp1-C1531A::TRP1*
K15544	*MATa Δsmc3::HIS3, leu2::SMC3-HA3::LEU2*
K16351	*MATa Δsmc3::HIS3, leu2::SMC3-HA3::LEU2, Δhos1::KanMX4*
K16353	*MAta UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, ECO1::kanMX-tetO2p-DHFRts-HA3-ECO1,Smc3-PK6::KanMx4::URA3*
K16354	*MAta UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244*
K16394	*MATalpha TRP1::P^{MET3}CDC20, SCC1-HA6::HIS3, SMC3-PK6::KanMx6, YEplac112-PGAL1TEV*
K16397	*MATa TRP1::P^{MET3}CDC20, SCC1(TEV220)-HA6::HIS3, SMC3-PK6::KanMx6*
K16465	*MATα TRP1::P^{MET3}CDC20, SCC1(TEV220)-HA6::HIS3, SMC3-PK6::KanMx6, Δhos1::KanMx4::ADE2, YEplac112-PGAL1TEV*
K16466	*MATa, ade2-101, Δhos1::KanMx4, CFIII (CEN3. L.YPH278) TRP1, SUP11*
K16470	*MATa ura3::3XURA3 tetO2X112, his3::HIS3tetR-GFP, Δhos1::kanMx4, TRP1::P^{MET3}CDC20*
K16472	*MATa ura3::3XURA3 tetO2X112, his3::HIS3tetR-GFP, TRP1::P^{MET3}CDC20*
K16546	*MATa, SCC1-MYC18::TRP1, SMC3-PK6::KanMX4, pGAL1-10-SCC1(R_{180}D, R_{268}D)-HA3::LEU2*
K16548	*MATa, SMC3-PK6, pGAL1-10-SCC1-HA3::LEU2*
K16653	*MATa SMC3-HA6::HIS3, dbf4::TRP1::SWI5p-DBF4*
K16655	*MATa SMC3-HA6::HIS3*
K16842	*MATa SMC3-HA6::HIS3, Δctf8::NaTMX*
K16845	*MATa SMC3-HA6::HIS3, Δctf4::NaTMX*
K17304	*MATa SCC1-HA6::HIS3, SMC3-MYC18::URA3, Δhos1::KanMx4*
K17305	*MATα esp1-1, Δhos1::KanMx4, pGAL1-10-ESP1::TRP1*
K17506	*MATa tetO2X224::URA3 (17.8 kb frm CEN5) , leu2::tetR-GFP::LEU2, Δhos1::kanMx4::ADE2, TRP1::P^{MET3}CDC20*
K17508	*MATa tetO2X224::URA3 (17.8 kb frm CEN5), leu2::tetR-GFP::LEU2, TRP1::P^{MET3}CDC20*
K17510	*MATa tetO2X224::URA3 (12.6 kb frm CEN5) ,leu2::tetR-GFP::LEU2, hos1Δ::kanMx4::ADE2, TRP1::P^{MET3}CDC20*
K17512	*MATa tetO2X224::URA3 (12.6 kb frm CEN5) ,leu2::tetR-GFP::LEU2, TRP1::P^{MET3}CDC20*

K17515	*MATa UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, Smc3-PK6::KanMx4::URA3, his3::GFP-LacI::HIS3, trp1::256lacO::TRP1, NatMx::P^{MET3}CDC20*
K17516	*MATa UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, Smc3-PK6::KanMx4::URA3, his3::GFP-LacI::HIS3, trp1::256lacO::TRP1, Δhos1::KanMx4::ADE2, NatMx::P^{MET3}CDC20*
K17376	*MATa UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, Smc3-PK6::KanMx4::URA3, ECO1::kanMX-tetO2p-DHFRts-HA3-ECO1 , his3::GFP-LacI::HIS3, trp1::256lacO::TRP1, NatMx::P^{MET3}CDC20*
K17382	*MATa UBR1::GAL1,10p-Glu-UBR1/CMVp-tTA(HIS3),LEU2::pCM244, Smc3-PK6::KanMx4::URA3, ECO1::kanMX-tetO2p-DHFRts-HA3-ECO1, his3::GFP-LacI::HIS3, trp1::256lacO::TRP1, Δhos1::KanMx4::ADE2, NatMx::P^{MET3}CDC20*
K17383	*MATa ura3::3XURA3 tetO2X112, his3::tetR-GFP::HIS3, TRP1::P^{MET3}CDC20, YEplac181-PGAL1HOS1*
K17384	*MATa ura3::3XURA3 tetO2X112, his3::tetR-GFP::HIS3, TRP1::P^{MET3}CDC20, YEplac181*
K17513	*MATa trp1::256LacO::TRP1, his3::LACI-GFP::HIS3, TRP1::P^{MET3}CDC20, SMC3-PK6::KanMx4, YEplac195-PGAL1HOS1*
K17514	*MATa trp1::256LacO::TRP1, his3::LACI-GFP::HIS3, TRP1::P^{MET3}CDC20, SMC3-PK6::KanMx4, YEplac195*
K17957	MATa SMC3-PK6::KanMx4, *TRP1::P^{MET3}CDC20, pGAL1-10-SCC1(R_{180}D, R_{268}D)-HA3::LEU2, his3::tetR-GFP::HIS3, YElac195*
K17955	MATa SMC3-PK6::KanMx4, *TRP1::P^{MET3}CDC20, pGAL1-10-SCC1(R_{180}D, R_{268}D)-HA3::LEU2, his3::tetR-GFP::HIS3, YElac195-Gal-Eco1*
K17987	*MATα trp1::256LacO::TRP1, his3::LACI-GFP::HIS3, NatMx4::P^{MET3}CDC20, Δhos1::KanMx4::ADE2, YEplac195-SMC1/SMC3*
K17988	*MATα trp1::256LacO::TRP1, his3::LACI-GFP::HIS3, NatMx4::P^{MET3}CDC20, Δhos1::KanMx4::ADE2, YEplac195*
K17989	*MATa trp1::256LacO::TRP1, his3::LACI-GFP::HIS3, NatMx4::P^{MET3}CDC20, YEplac195-SMC1/SMC3*
K17990	*MATa trp1::256LacO::TRP1, his3::LACI-GFP::HIS3, NatMx4::P^{MET3}CDC20, YEplac195*
K17966	*MATa SMC3-HA6::HIS3, Δhos1::KanMx4*
K17996	*MATa SMC3-HA6::HIS3, Δhos2::KanMx4*
K17997	*MATa SMC3-HA6::HIS3, Δhos3::KanMx4*
K17998	*MATa SMC3-HA6::HIS3, Δhst1::KanMx4*
K17999	*MATa SMC3-HA6::HIS3, Δhst2::KanMx4*
K18000	*MATa SMC3-HA6::HIS3, Δhst3::KanMx4*

K18001	*MATa SMC3-HA6::HIS3, Δhst4::KanMx4*
K18002	*MATa SMC3-HA6::HIS3, Δsay1::KanMx4*
K18003	*MATa SMC3-HA6::HIS3, Δrpd3::KanMx4*
K18004	*MATa SMC3-HA6::HIS3, Δhda1::KanMx4*
K18005	*MATa SMC3-HA6::HIS3, Δcda1::KanMx4*
K18006	*MATa SMC3-HA6::HIS3, Δhos2::KanMx4, Δhos1::KanMx4::ADE2*
K18007	*MATa SMC3-HA6::HIS3, Δhst2::KanMx4, Δhos1::KanMx4::ADE*
K18008	*MATa SMC3-HA6::HIS3, Δhst3::KanMx4, Δhos1::KanMx4::ADE2*
K18009	*MATa SMC3-HA6::HIS3, Δhst4::KanMx4, Δhos1::KanMx4::ADE2*
K18010	*MATa SMC3-HA6::HIS3, Δhst1::KanMx4, Δhos1::KanMx4::ADE2*
K18011	*MATa SMC3-HA6::HIS3, Δhda1::KanMx4, Δhos1::KanMx4::ADE2*
K18012	*MATa SMC3-HA6::HIS3, Δhos3::KanMx4, Δhos1::KanMx4::ADE2*
K18013	*MATa SMC3-HA6::HIS3, Δsay1::KanMx4, Δhos1::KanMx4::ADE2*
K18014	*MATa SMC3-HA6::HIS3, Δrpd3::KanMx4, Δhos1::KanMx4::ADE2*
K18015	*MATa SMC3-HA6::HIS3, Δcda1::KanMx4, Δhos1::KanMx4::ADE2*
K18016	*MATa SMC3-HA6::HIS3, Δcda2::KanMx4, Δhos1::KanMx4::ADE2*
K18017	*MATa SMC3-HA6::HIS3, Δcda2::KanMx4*
K18018	*MATa SMC3-HA6::HIS3, Δsir2::KanMx4*